Cell Cycle Control and Cancer

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Multiple genetic changes occur during the evolution of normal cells into cancer cells. This evolution is facilitated in cancer cells by loss of fidelity in the processes that replicate, repair, and segregate the genome. Recent advances in our understanding of the cell cycle reveal how fidelity is normally achieved by the coordinated activity of cyclin-dependent kinases, checkpoint controls, and repair pathways and how this fidelity can be abrogated by specific genetic changes. These insights suggest molecular mechanisms for cellular transformation and may help to identify potential targets for improved cancer therapies.

Cancer cells differ from normal cells in many important characteristics, including loss of differentiation, increased invasiveness, and decreased drug sensitivity. These differences do not arise simply from uncontrolled cellular growth but rather from a process of cellular evolution. The increased incidence of cancer as a function of age has long been interpreted to suggest that multiple genetic changes are required for tumorigenesis (*1*, *2*), an interpretation borne out by recent systematic analysis of genetic changes during the evolution of colon cancer cells (*2*, *3*).

Peter Nowell suggested in 1976 that cancer cells might have mutations that result in genetic instability and thereby accelerate cellular evolution (*4*). Subsequent work has verified this view. For example, mutations in DNA repair genes [mismatch repair (*5*) and excision repair (*6*)] predispose carriers to cancer, presumably by increasing genomic instability. A number of other rare hereditary syndromes [Fanconi's, Bloom's, Werner's, ataxia telangiectasia (AT)], the genetic origins of which are not yet understood, are characterized by sensitivity to DNA-damaging agents, a high frequency of chromosomal rearrangements, and a predisposition to cancer (*6*). Karyotypic alterations, including whole chromosome loss or gain, ploidy changes, and a variety of chromosome aberrations are common in cancer cells (Fig. 1) (*3*, *7*).

Recent work has identified another category of genes that, when mutated, increase genetic instability and accelerate cellular evolution. These genes encode components of cell cycle checkpoints, which are positions of control that ensure the order of events in the cell cycle and that integrate DNA repair with cell cycle progression. This review will focus on the role of cell cycle checkpoints in cancer cell evolution and their potential impact on cancer prevention and treatment. But to understand the role of checkpoints in cancer, we need first to consider their role in the normal cell cycle.

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Control of Cell Cycle Progression

Completion of the cell cycle requires the coordination of a variety of macromolecular syntheses, assemblies, and movements (Fig. 2) [for a recent review, see (*8*)]. The chromosomes must be replicated, condensed, segregated, and decondensed. The spindle poles must duplicate, separate, and migrate to opposite ends of the nucleus. In metazoa, the nuclear membrane is disassembled and reassembled, the spindle is assembled and disassembled, and the cell membranes invaginate to complete cytokinesis. Coordination of these complex processes is thought to be achieved by a series of changes (phase transitions) in the cyclin-dependent kinases (CDKs). The active forms of the CDKs are a complex of at least two proteins, a kinase and a cyclin, and often contain other proteins of poorly understood function. These complexes undergo changes in the kinase and cyclin components that are believed to drive the cell from one stage of the cell cycle to another. According to this paradigm, cell cycle stage is determined by the constellation of proteins activated or inactivated by phosphorylation as a result of the activity of the CDKs during that stage.

The succession of CDK changes is best understood in the yeast *Saccharomyces cerevisiae*, in which a single kinase component, the product of the *CDC28* gene interacts successively with a series of transiently expressed cyclins (*9*). Each of the cyclin genes (except *CLN3*) is transcribed for a brief period during the cell cycle, the messenger RNA (mRNA) is translated, and the protein is then rapidly degraded; thus, each cyclin protein is present during only one stage of the cell cycle. In mammalian cells, a succession of kinase subunits (CDK4, CDK2, and CDC2) is expressed along with a succession of cyclins (D, E, A, and B) as the cells progress from G_1 to mitosis (*10*). CDK4 (complexed with one of several D cyclins) functions early, probably in response to growth factors; CDK2 (probably complexed to cyclin E or cyclin A or both) is essential for DNA replication; and CDC2 (complexed with cyclins A and B) is essential for mitosis. Additional CDKs (*11*) and cyclins will undoubtedly be added to this list. The functional homology among eukaryotic CDKs and cyclins is striking. Most of the mammalian CDKs and cyclins can functionally replace the corresponding yeast proteins, and the same is true for enzymes that regulate the activity of the kinases.

The passage of cells from one stage of the cell cycle to another is tightly regulated by a wealth of controls that act on the transcription of cyclin genes, the degradation of cyclin proteins, and the modification of the kinase subunits by phosphorylation (*9*, *12*). A number of positive and negative feedback loops also contribute to cell cycle progression (*9*). Wherever pertinent information is available, these controls appear to be present in yeast, in the fruit fly *Drosophila*, and in vertebrates, although variations exist in the extent to which particular controls are used in different growth conditions or developmental stages.

Negative controls on cell cycle progression are exerted during development, differentiation, senescence, and cell death. These negative controls may play an important role in preventing tumorigenesis. In many cases, arrest of cell proliferation takes place under circumstances in which the integrity of the genome has been compromised, and failure to arrest proliferation would release cells with highly unstable genomes that could evolve into cancer cells. Such circumstances might include, for example (i) senescence, in which telomeres are lost or become short, and unstable dicentric chromosomes are formed; (ii) "programmed cell death" or apoptosis, in which DNA-degrading nucleases are unleashed; and (iii) immune cell development, in which requisite immunoglobulin and T cell receptor gene rearrangements require double-strand DNA breaks. These are all programmed events that may include an arrest of proliferation.

Cells also have the capacity to arrest cell cycle progression when damage is induced by unprogrammed extrinsic events (*13*), such as exposure to inhibitors of DNA replication or spindle assembly or to agents that physically damage DNA. These perturbations result in arrest of cell cycle progression at a specific stage. For example, when

DNA replication is inhibited by hydroxyurea, the cell arrests in early S phase and does not undergo mitosis. This dependence of mitosis on prior completion of DNA replication is due to the action of specific gene products (*14–16*). Inactivation of these genes by mutation relieves the cell of this dependence, and such mutants will enter mitosis with incompletely replicated DNA. The genes that establish dependence in the cell cycle constitute checkpoints (*13*).

How do checkpoints arrest cell cycle progression? The simplest model is that the checkpoint prevents the next CDK phase transition until the stalled process can be completed or until repair is effected. Although still unproven, this concept is supported by evidence from a variety of experimental systems. For example, in the yeast *Schizosaccharomyces pombe*, mutations in *CDC2* can eliminate the dependence of mitosis on the completion of DNA replication (*17*). In nuclear extracts from *Xenopus* oocytes, if DNA replication is blocked, then removal of the inhibitory phosphate from the CDC2 kinase is also blocked, so that mitosis cannot be activated (*18*). The arrest of mammalian cell growth by contact inhibition or transforming growth factor–β (TGF-β) is mediated through an inhibitory protein that prevents assembly and activation of cyclin E–CDK2 complexes (*19, 20*). Three inhibitors of cyclin-CDK activity have been identified in mammalian cells: p16, p21, and p27 (*20–23*), and two have been identified in *S. cerevisiae*: FAR1 and p40 (*24*). These inhibitors all appear to block cell cycle progression in G_1, although they may have as yet undetected effects on other cell cycle stages. They are activated by different physiological signals and act on different CDK-cyclin complexes (Fig. 3).

Checkpoints and Tumorigenesis

Although our knowledge of cell cycle checkpoints is still incomplete, it is clear that many such control points exist within the cell cycle and that they play a major role in maintaining the integrity of the genome. The three cellular components involved in genome transmission—the DNA, the spindle, and the spindle pole—are all under surveillance during their replication and segregation. As discussed below, defects in surveillance of each of these components result in forms of genetic instability that characterize precancerous and cancerous cells. Defects in surveillance of the DNA could be responsible for chromosomal rearrangements such as deletions, amplifications, and translocations (Fig. 4). Defects in spindle surveillance could lead to mitotic nondisjunction, producing whole chromosome loss or gain, and defects in surveillance of the spindle poles could lead to changes in the ploidy of the genome. These three categories of genomic change, chromosomal rearrangements, aneuploidy, and polyploidy, are all common during cancer cell evolution.

At least two checkpoints detect DNA damage: one at the G_1-S transition and one at the G_2-M transition (Fig. 5). In addition, surveillance of telomeric sequences as they are lost during senescence may be important in signaling somatic cells to stop proliferation (*25, 26*). The checkpoint controlling entry into S phase prevents the cell from replicating damaged DNA and is currently best understood in mammalian cells. Cells with DNA damage rapidly increase p53 protein levels by a posttranscriptional mechanism (Fig. 3) (*27*). Induction of p53 results in transcriptional activation of p53-dependent genes such as *GADD45* (*28*), *MDM2* (*29*), and *WAF-1/CIP1/SDI1* (*21, 22*) and either cellular arrest in G_1 or apoptosis. Likely targets for the G_1-S checkpoint are CDKs containing the cyclins expressed early in the cell cycle, such as cyclins D, E, and A. Cyclin E–CDK2 and cyclin A–CDK2 activities have recently been shown to be inhibited by ionizing radiation in a p53-dependent manner, presumably through transcriptional activation of p21 (*30*).

Considerable experimental evidence supports the view that loss of the G_1-S checkpoint can lead to genomic instability, inappropriate survival of genetically damaged cells, and the evolution of cells to malignancy.

1) The fact that *p53* is commonly mutated in a wide variety of human cancers (*31*) suggests that abnormalities in the G_1-S checkpoint are important in tumorigenesis.

2) Although rare in normal cells, aneuploidy and gene amplification are common in *p53* mutant cells (*32, 33*), indicating that defects in this signal transduction pathway result in genetic instability.

3) The gene products of certain DNA cancer viruses [SV40, human papilloma virus (HPV), and adenovirus] alter the function of several cellular proteins, including p53 and the retinoblastoma susceptibility gene product Rb, and can affect cell cycle checkpoint function (*33, 34*). HPV infection, for example, has already been tightly linked to the development of certain human cancers, particularly cervical carcinoma (*35*). Furthermore, expression of SV40 T antigen, which binds to and functionally inactivates p53 (*36*), induces karyotypic instability (*37*) before neoplastic transformation.

4) Patients with AT, a hereditary syndrome that predisposes to cancer, have a

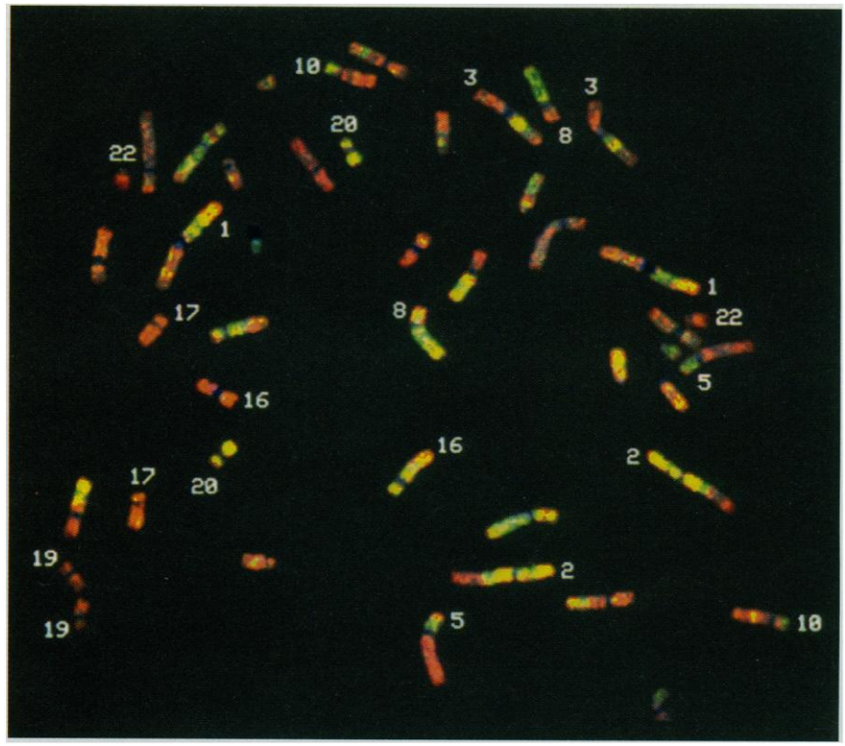

Fig. 1. Visualization of chromosomal abnormalities in cancer cells. Shown are the results of comparative genomic hybridization in which a mixture of bladder cancer cell DNA and normal bladder cell DNA were differentially labeled and hybridized to a normal karyotype. Cancer DNA that is present in increased copy number is green and that present in decreased copy number is red. Color contrast from the original photo was digitally enhanced with Adobe Photoworkshop (*101*). (This is a previously unpublished photograph courtesy of A. Kallioniemi, O. Kallioniemi, and F. Waldman.)

markedly increased incidence of cancers, particularly lymphoblastic lymphomas (*38*). The gene products that are defective in this disease are required for optimal induction of p53 after exposure to ionizing radiation (*28, 30, 39*). Unlike wild-type cells, cells defective for *p53* or the *AT* genes continue to enter S phase after irradiation (*27, 40*), with an increased potential for genomic instability (*32*). Moreover, there is some indication that individuals heterozygous for *AT* mutations (*41*) may have an increased incidence of breast cancer, which suggests that a cancer susceptibility phenotype can result from even subtle defects in this surveillance pathway.

5) Evidence supporting a role for the p53-dependent G_1-S checkpoint pathway in the development of human cancers has come from studies on adenocarcinomas in esophageal epithelium (*42*). The histologic, cell cycle, and genetic changes that occur during the progression of "Barrett's epithelium" have been carefully mapped through serial biopsies. Most cells in the upper gastrointestinal tract are normally in a nonproliferative stage. In Barrett's epithelium, there is an increased fraction of cells in G_1 early in disease progression. More advanced stages of neoplastic progression are characterized by clones of cells exhibiting an increased fraction of cells in S phase or in G_2. In esophageal cancers exhibiting loss of heterozygosity at chromosome 17p (presumably reflecting *p53* gene loss), this change typically precedes the development of aneuploidy and the emergence of a malignant histologic phenotype. Thus, at least in this cancer type, the paradigm seems to be borne out: Initially, cellular damage leads to cell cycle arrest, but when loss of a cell cycle checkpoint occurs, genetic instability develops and the cells progress to a malignant phenotype. Abnormal expression of cyclins D, E, and A in some cancers (*43, 44*), as well as altered associations of various cyclin-CDK proteins in p53-deficient cells (*45*), may provide additional mechanisms of abrogating the G_1-S checkpoint during tumorigenesis.

In some tissue types or under certain physiologic conditions, p53 induction by DNA damage appears to trigger apoptosis rather than G_1 arrest (*46, 47*). In these instances, loss of apoptotic signals may contribute to genomic instability and tumorigenesis by loss of a mechanism for eliminating cells with genetic damage. This could occur early in cancer progression, leading to genetic instability by survival of genetically damaged cells; alternatively, it could occur later in tumorigenesis and contribute directly to survival of cells in inappropriate physiological situations (*48, 49*). This mechanism is important for negative selection in thymic tissue and, when aberrant, may contribute to the development of lymphoblastic lymphomas. Further support for this concept comes from the fact that the lymphoma-associated oncogene *BCL2* can block p53-mediated apoptosis after irradiation of thymocytes and other cell types (*50*). In addition, transforming oncogenes such as *c-MYC* and adenovirus *EIA* can simultaneously stimulate cellular proliferation and apopotosis (*51*). Thus, programmed death of cells is affected by many of the same gene products controlling cell cycle progression, resulting both in increased genomic instability and in increased survival of these abnormal cells.

The G_2-M transition is prevented by DNA damage and by incompletely replicated DNA (Fig. 5). This checkpoint prevents chromosome segregation if the chromosome is not intact. Genetic studies in *S. cerevisiae* (*16, 52*) and *S. pombe* (*15, 53*) have identified a number of genes necessary for this control. In *S. cerevisiae*, the *RAD9*, *RAD17*, *RAD24*, *MEC1*, *MEC2*, and *MEC3* genes prevent mitosis in the presence of DNA damage or if replication is blocked in late S phase. The *MEC1* and *MEC2* genes also prevent mitosis if replication is blocked in the early S phase. One double-strand break in the DNA will activate this checkpoint and prevent the cell from undergoing mitosis (*54*). Some of the *S. cerevisiae* genes show sequence homology to the *S. pombe* genes, which indicates that this pathway evolved early and is likely to be present in human cells as well.

Few gene products that control the G_2-M transition have been identified in mammalian cells. Several observations suggest that defects in the regulation of these

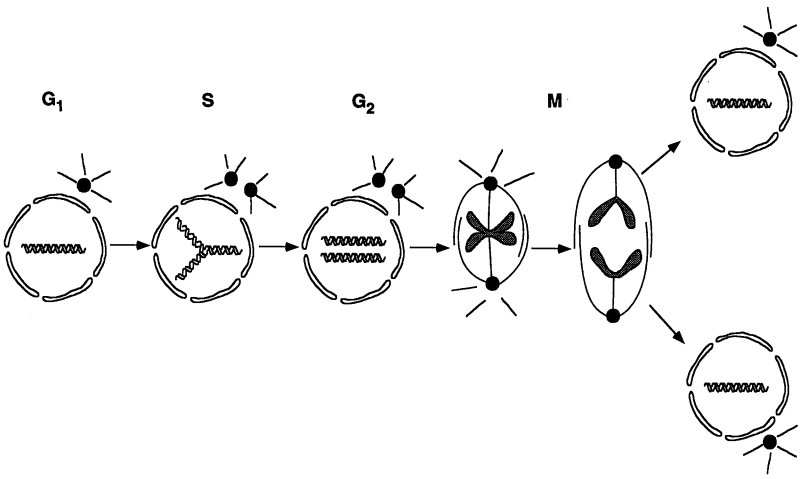

Fig. 2. The cell cycle. Some of the important events of the eukaryotic cell cycle are shown: centrosome (spindle pole body) duplication and segregation, DNA replication, condensation and segregation, spindle formation and elongation, and nuclear envelope breakdown and reformation.

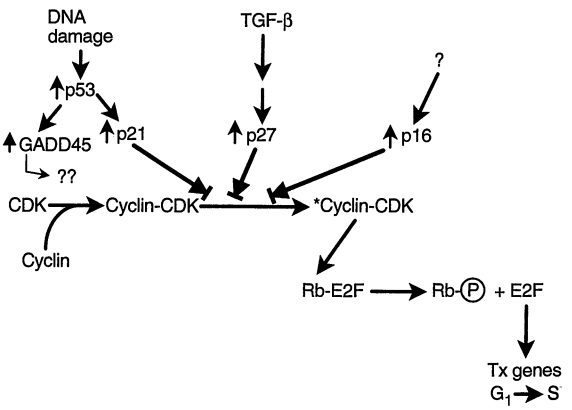

Fig. 3. Schematic representation of the roles of p21, p16, and p27, proteins that inhibit activation of cyclin-CDK complexes. Cyclins, whose levels vary throughout the cell cycle, associate with the stably expressed CDKs. These cyclin-CDK complexes are activated (asterisk) (*8*), and the kinase activities are required for progression to the next step of the cell cycle. In the case of progression from G_1 into S phase, one apparent target of the activated kinase is the Rb protein. Rb is bound to the transcription factor E2F during G_1, but upon phosphorylation, E2F is released and activates the transcription (Tx) of genes required for transition into S phase. A family of Rb-like molecules (Rb, p107, p130, and p300) and a family of E2F-like transcription factors (E2F-1, -2, -3, and -4 and DP-1 and -2) may function similarly or in combination. Induction of p21 (also known as WAF1 or CIP1) appears to be at least partially dependent on p53 (*21, 22*) and is induced when cells are exposed to DNA-damaging agents (*21, 22*). p21 appears to inhibit all of the cyclin-CDK complexes. The role of GADD45 in mediating p53-dependent responses to DNA damage has not yet been elucidated. p27, which is induced by TGF-β and by cell-cell contact, inhibits one or more cyclin-CDK complexes (*20*). p16 specifically inhibits cyclin D_1-CDK4 activation. The physiologic inducers of p16 have not been elucidated.

transitions may also be important in human tumorigenesis, although the data are much less compelling than for the G_1-S transition. Non-neoplastic cells from individuals with familial cancer predisposition display a higher than average frequency of mitotic chromosomal breaks after irradiation (*55*). Cells from AT patients undergo "suboptimal arrest" after irradiation in G_2 (*56*). A number of cell lines derived from human cancers exhibit reduced G_2 delay after DNA damage (*57*). Altered expression of cyclins A, B, and CDC2, all potential targets of mitotic checkpoint controls, occurs in some cancers (*44*).

Many environmental carcinogens are DNA-damaging agents. The G_1-S and G_2-M checkpoints may be important in protecting cells from exogenous sources of DNA damage as discussed above. However, DNA damage can also be caused by intrinsic cellular processes, including gene rearrangement during development, cell senescence, and apoptosis. In cases where damage is generated by intrinsic processes, negative controls over the proliferation of damaged cells are likely to be important in preventing the evolution of cancer cells.

Cancer incidence in humans increases exponentially with age (*58*). Many theories have been put forth to explain this association, including increased DNA damage due to accumulated exposure to DNA-damaging agents or to decreased DNA repair capacity (*58, 59*). Although it is not clear that the decrease in cellular proliferation (senescence) in vitro accurately reflects in vivo aging, studies of senescing cells in culture have revealed a potentially important source of intrinsic DNA damage. Normal human fibroblasts do not express telomerase, the enzyme that replicates the repeated sequences at the ends of chromosomes; thus, telomere length decreases as cells proliferate. It has been suggested that senescence occurs as a result of the loss of telomeric sequences (*25, 26*) and that chromosome ends with shortened telomeres may activate a checkpoint pathway that inhibits cell proliferation (*26*). Senescing cells exhibit an increased number of chromosomal aberrations, many of which appear to involve telomere-telomere associations (*60*). Thus, the normal senescent program may generate chromosome instability. One gene thought to be necessary for the arrest of proliferation in senescent cells is *SDI1* (*61*), recently shown to be identical to *WAF1/CIP1* (*21, 22*), whose product inhibits CDKs. Loss of SDI1 or of other proteins important for arresting division in cells that have lost telomeric sequences could lead to a cascade of genomic instability.

Breaks are introduced into DNA during the rearrangement of immunoglobulin and T cell receptor genes. It is likely that proteins yet to be identified inhibit cell cycle progression during these events. Genes coding for these proteins provide additional potential targets for mutations that would generate chromosome instability, and such mutations could be important in the etiology of lymphomas and leukemias, in which errors in gene rearrangement generate oncogenes. Interestingly, lymphoblastic lymphomas are the primary cancers seen in both AT patients (*38*) and mice with disrupted *p53* alleles (*62*). Endonucleases degrade DNA during apoptosis. Similar considerations apply here. Escape from cell division arrest by mutation of genes that negatively control division during apoptosis could lead to the proliferation of cells that have embarked on a program of genetic instability.

Improper functioning of the mitotic spindle at metaphase can arrest cell cycle progression (Fig. 5). For example, chromosome segregation at the metaphase-anaphase transition is prevented if one or more chromosomes are not yet congressed at the metaphase plate (*63*). Moreover, the initiation of a new cell cycle is prevented if mitosis was not completed in the previous cell cycle because of inhibition of microtubule assembly (*64*). *Saccharomyces cerevisiae* encodes at least six genes that prevent the cell from initiating a new cell cycle in the presence of microtubule poisons: *MAD1*, *MAD2*, *MAD3*, *BUB1*, *BUB2*, and *BUB3* (*65, 66*).

The centrosome (or spindle pole) transitions have been less intensely studied, in part because no specific inhibitors exist that directly block stages of the centrosome cycle. However, the phenotypes of mutants defective for spindle pole duplication in yeast suggest that a failure of spindle pole duplication arrests the completion of mitosis through a checkpoint control (Fig. 5) (*67*).

Little is known about the mammalian proteins that control the G_2-M transition or that monitor the spindle or spindle poles. However, several lines of evidence suggest that defects in the regulation of these processes could be important in human tumorigenesis. First, cells from young cancer patients have been reported to exhibit increased resistance to antimicrotubule agents relative to cells from healthy children (*68*). Second, expression of SV40 large T antigen in murine pancreatic tissue produces abnormalities in centriole number and segregation that subsequently cause chromosomal instability (*69*). Finally, the c-*mos* protooncogene, a regulator of meiotic metaphase, produces polyploidy when it is expressed abnormally in mitotic cells, as it is during tumorigenesis (*70*).

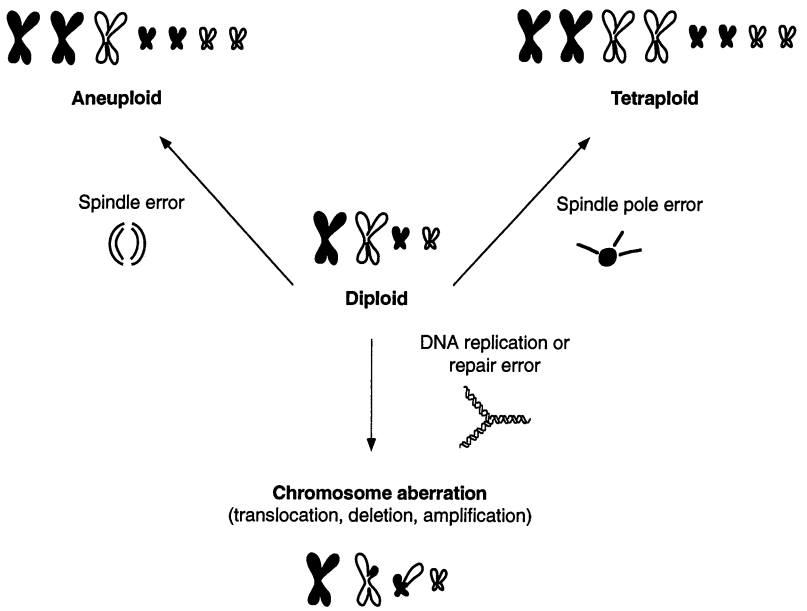

Fig. 4. Origins of genomic changes. Spindle errors produce aneuploidy, spindle pole errors produce whole genome changes, and replication errors produce chromosome aberrations.

Cell Cycle Control and Cancer Therapy

A limiting factor in human cancer therapy is its toxicity to normal tissues. If it were possible, for example, to irradiate the whole body with 12,000 centi-Gray (cGy) of ionizing radiation, every cancer would he curable; unfortunately, such doses would also be lethal to the host. Thus, the goal in cancer therapy is to kill cancer cells while sparing normal tissues. Currently, two strategies are used for targeting chemotherapy to cancer cells: localized delivery of high doses of cytotoxic agents and application of agents that kill dividing cells more effec-

tively than nondividing cells. What is lacking in most current treatment regimes is therapy that is based on physiological differences in the responses of normal and cancer cells to antineoplastic agents.

Most of the antineoplastic agents now in use work by directly damaging cellular DNA, inhibiting synthesis of or incorporation of precursors into DNA, inhibiting the mitotic apparatus, or inhibiting topoisomerases. The success of these agents in selectively killing cancer cells appears to vary primarily as a function of the cancer type. Some cancers are sensitive to these agents and are curable (for example, childhood acute lymphoblastic leukemia and germ cell cancers), whereas others are relatively resistant and are not usually curable (for example, colon carcinoma). This variability probably reflects cell-type-specific responses to DNA-damaging agents in cancer cells arising from different tissues. For example, the likelihood that a particular cell type will undergo apoptosis may be a major factor determining the response of a given cancer to chemotherapy.

Cell cycle checkpoints offer a new set of potential targets for chemotherapeutic compounds (*71*). In this context, several important properties of checkpoints merit consideration.

1) Checkpoints are signal transduction systems. They must receive a signal, then amplify and transmit that signal to other components that regulate the cell cycle. Double-strand DNA breaks (*54*, *72*, *73*), unexcised ultraviolet light–induced dimers in DNA, and centromeres not engaged by the spindle (*63*, *74*) are potential signals. The fact that there are at least six components for each of the checkpoint controls that have been studied genetically suggests the presence of transmission cascades. Thus, each such pathway provides multiple targets for therapeutic intervention.

2) Many checkpoints are nonessential in unperturbed cells. For several checkpoints, deletion of certain genes in the pathway has little or no effect on the growth rate of cells that are not exposed to damaging agents (*62*, *66*, *75*). However, other genes in these pathways may be essential (*16*, *65*), either because they encode essential components of the cell cycle machinery that emit or receive the signal or because their products have more than one cellular function. For nonessential pathways, potentially useful therapeutic agents targeted to checkpoint controls will only be detected by their synergy with other agents that damage or perturb the cell. Moreover, these antagonists may have little or no toxicity by themselves.

3) Checkpoints ensure the fidelity of genomic replication and segregation. For example, *S. cerevisiae* cells defective for the G_2-M checkpoint that monitors double-strand DNA breaks exhibit a 20-fold increase in the rate of chromosome loss in the absence of any extrinsic perturbation (*75*), and cells defective for the G_2-M checkpoint that responds to spindle defects exhibit a 15- to 30-fold increase in chromosome loss (*65*). These results imply that there is a biologically significant level of spontaneous damage that requires checkpoint control in order for cells to maintain the high fidelity of chromosome transmission. This stochastic damage may be important in driving the evolution of cancer cells with compromised checkpoints. Therefore, restoration of compromised checkpoints could slow cancer cell evolution even in the absence of exogenous sources of DNA damage.

4) Many signal transduction systems, including checkpoint controls, exhibit adaptation—that is, in the presence of a constant stimulus, their response diminishes with time. As a consequence, the cell may proceed through the cell cycle even though the original perturbation has not been removed or cannot be repaired. For example, yeast cells that retain an unrepaired double-strand DNA break eventually proceed into mitosis after a delay in G_2 (*54*). Genetic lesions that increase the ability of the cell to adapt could accelerate genomic evolution. Alterations in expression of the p53 binding protein MDM2 could result in adaptation of the G_1 checkpoint (*29*, *76*). Moreover, inhibition of components involved in adaptation provide potential therapeutic targets for the restoration of defective checkpoints as a means to slow the evolution of precancerous cells.

5) Checkpoint activation may induce a variety of cell responses, including cell death. The checkpoint controlling entry into S phase in mammalian cells includes p53, a transcription factor that can induce many genes. One of the functions under the control of this pathway is apoptosis. Irradiation is far less toxic to p53 mutant lymphocytes than to those with wild-type p53 (*46*). Thus, the expected deleterious effects of mutant p53 on cells entering S phase with damaged DNA is more than offset by the fact that the p53 wild-type cells undergo apoptosis when their DNA is damaged. The role of checkpoints in activating apoptosis is likely to be influenced by cell type and by the nature of the proliferation signals or damage to which the cell is responding. Restoration of defective checkpoints could restore the apoptotic response of cancer cells and increase their sensitivity to DNA-damaging agents. The components of the apoptotic response may be useful therapeutic targets if apoptosis could be activated in the absence of DNA damage. It may be possible to achieve specificity for certain types of cancer cells, because it is clear that not all cells respond to the same apoptotic signals (*77*, *78*).

Because many cancers are not curable with currently available compounds, there is great incentive to identify other useful targets in cancer cells. Many of the new insights into the molecular controls of cell cycle progression and the genetic and biochemical distinctions between normal and cancer cells in these controls provide a use-

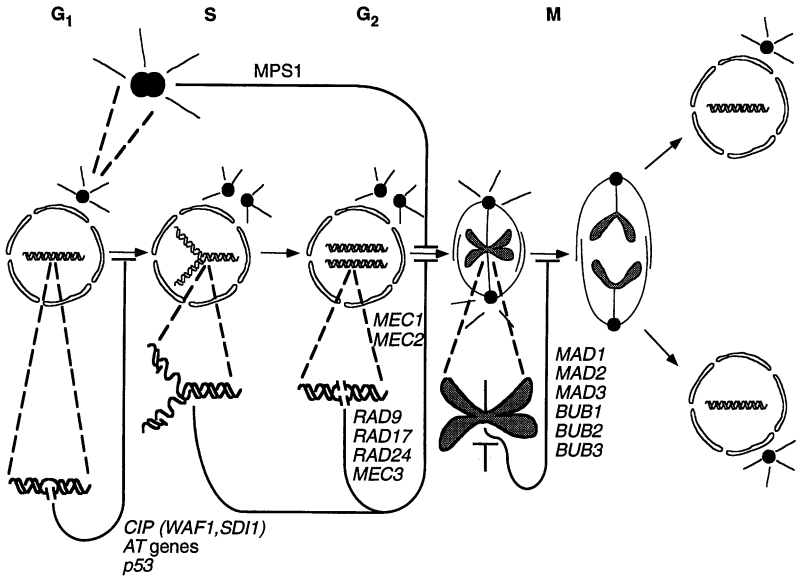

Fig. 5. Cell cycle checkpoints. A composite map showing some of the cell cycle checkpoints. Defects in spindle pole duplication can block mitosis. Nicks in DNA can delay the onset of replication. Arrested DNA replication and DNA double-strand breaks can block mitosis. Chromosomes unattached to the mitotic spindle can block anaphase. The diagram is incomplete for the sake of simplicity. Notable omissions include a role for the AT genes in the G_2-M transition and a role for some of the genes in the RAD9 pathway in the G_1-S transition.

ful conceptual framework in which to identify new potential targets for antineoplastic therapies and methods for identifying agents that affect these new targets. The development of new chemotherapeutic and prevention strategies for cancer treatment will require a greater understanding of the molecular components of cell cycle checkpoints and the DNA repair machinery than we now have. Nevertheless, some predictions can be made. We assume that strategies that kill cells are likely to be more effective than those that merely arrest proliferation and that cancer cells are frequently missing cell cycle checkpoints or DNA repair functions or both. Cancer cell survival after chemotherapy or radiotherapy will depend on the specific checkpoints or repair pathways that have been lost, leading either to greater susceptibility to these agents when the repair of damage is most important for survival or to greater resistance when the apoptotic response is more important. We can anticipate a time when it will be possible to characterize tumors individually for their checkpoint and repair status and thereby predict their response to particular therapies. This knowledge, coupled with new drugs that inhibit or activate specific checkpoints, may allow the design of therapeutic strategies that are very effective.

In terms of specific implementation of these principles, we discuss below several strategies that derive from our emerging understanding of the p53-dependent G_1-S checkpoint in mammalian cells and the G_2-M checkpoint in yeast. We emphasize that these examples are speculative and are based largely on ideas that many other workers in the field have presented.

Genetic studies in *S. cerevisiae* and *S. pombe* have identified numerous genes that are necessary for arrest in G_2 after ionizing irradiation. Defects in any one of these genes lead to a marked increase in cell death after exposure to ionizing radiation (*14*) or lead to other forms of DNA damage. The mammalian homologs of these genes, if and when they are identified, will be logical targets for inhibition in order to increase cancer cell kill after exposure to certain antineoplastic therapies. Caffeine, although neither potent nor specific, prevents the arrest of cells in G_2 after DNA damage and increases the cytotoxicity of DNA damage (*47, 79*). For relatively localized cancers, systemic inhibition of a checkpoint should have no effect on cells not simultaneously exposed to the DNA-damaging agents; thus, the effectiveness of local irradiation of cancers or local delivery of cytotoxic agents could be facilitated by the use of such compounds. Cells from individuals with AT are defective in G_1, S, and G_2 arrests after ionizing irradiation and are extremely sensitive to the cytotoxic effects of irradiation (*40, 55, 80*). At least one of the genes responsible for this disease complex has been localized to chromosome 11q23 (*81*), but has not yet been identified. Conceivably, the AT gene product or products would be logical targets for drug design.

Some cell types, such as lymphocytes, undergo apoptosis rapidly in response to cytotoxic treatments, whereas others appear to be relatively resistant to this response. One feature that may distinguish the cancer types that can and cannot be cured with chemotherapy is the capacity of the curable cancers to undergo rapid apoptosis in response to cytotoxic agents. Some of the same gene products that control G_1-S cell cycle progression are also involved in controlling apoptosis (*51, 77, 82, 83*). Moreover, it has been argued that apoptosis occurs as a result of conflicting positive and negative growth signals (*83*), which suggests the possibility of additional links between gene products controlling apoptosis and the cell cycle. A few gene products have been identified that are directly involved in the apoptotic pathway, and many more are likely to be found. All of these provide targets for manipulating cell survival outcomes after exposure to cytotoxic agents. The Bcl-2 protein protects cells from apoptosis mediated by Bax homodimers by heterodimerizing with the Bax protein (*84*). Characterization of other Bcl-2–like or Bcl-2–interactive molecules, such as Bcl-xL and Bcl-xs (*85*) and Mcl-1 (*86*), similarly provide potential attractive targets for improving therapy. The protease ICE (*87*), which is homologous to the *Caenorhabditis elegans* gene *ced-3*, is necessary for apoptosis in certain situations, providing another potential target. Although some cell types (such as fibroblasts) use the ability of p53 to induce DNA damage to mediate an arrest in G_1, other cell types (such as thymocytes) use this p53 signal to initiate an apoptotic signal. It is clear that loss of p53 function in cells that initiate apoptosis (*48, 83, 88*) produces resistance to cytotoxic treatments. Thus, in cancer cells with wild-type *p53*, a therapeutic goal would be to direct the cells to apoptosis rather than to cycle arrest. In cancer cells with mutant *p53*, induction of apoptosis would have to be accomplished by manipulation of a biochemical event that is normally downstream of p53 in this signaling pathway.

Elucidation of the molecular controls of cell cycle progression may also provide novel ways of selecting cytotoxic agents that are based on the genotype of the cancer cell. For example, cancer cells devoid of Rb should have an increased level of unbound E2F-1 (Fig. 3), which theoretically would result in increased transcription of certain genes such as those encoding dihydrofolate reductase (DHFR) (*89*), thymidylate synthetase (TS), ribonucleotide reductase, and thymidine kinase. Because of the increased expression of DHFR, these cells might be relatively resistant to methotrexate. By comparison, 5-fluorouracil or another TS inhibitor might be more effective, not only because it functions downstream of DHFR, but also because it acts by binding TS and inhibiting its activity. Such a scenario might be particularly useful in a cancer such as osteosarcoma, in which *Rb* alleles are frequently deleted and in which methotrexate is a commonly used antineoplastic agent. Examples of relative methotrexate resistance correlating with loss of Rb function have been reported (*90*).

Novel gene products or overexpressed gene products specific to certain cancers provide other potential targets for cancer-specific therapy. For example, the chimeric bcr-abl tyrosine kinase (*91*) and the chimeric AML-1 transcription factor (*92*) are not present in normal tissues but arise from cancer-specific chromosomal translocations in chronic myelogenous leukemias and acute myeloid leukemias, respectively. In most cases, these gene products contribute to altered cell cycle control or altered apoptosis in the malignant cells; thus, targeting such cancer-specific gene expression would affect many of the same control pathways discussed above. The recent demonstration of telomerase expression in cancer cells but not in normal somatic cells, and the potential dependence of cancer cells on the telomerase activity for viability, makes the telomerase enzyme another attractive target for specific anticancer therapy (*93*). Loss of telomeres would probably activate the G_2-M checkpoint, cell cycle arrest (*54*), and possibly apoptosis.

Cell Cycle Control and Cancer Prevention

If development of genomic instability contributes to cellular transformation and cancer progression, then strategies that reduce instability could reduce the incidence or rate of cancer development. Understanding the molecular events that lead to genomic instability, including components involved in damage susceptibility, repair, cell cycle control, and apoptosis will be required for development of rational prevention strategies. p53 is currently the best-characterized cell cycle control protein linked to genetic instability and thus is the focus of the strategies suggested here.

Mutations in *p53* appear to occur relatively early in certain human cancer models and may be present in dysplastic, but nonmalignant, lesions of the bronchial epithelium (*94*). Exposure of such cells to further DNA-damaging agents might be expected to result in an increased frequency of genet-

ic aberrations. Prevention of further DNA damage, such as by cessation of smoking, could be critical at this stage. Not all DNA damage is caused by exposure to exogenous agents; exposure to "naturally occurring" oxidative damage (*95*) may also drive a premalignant cell to malignancy. Thus, prevention of oxidative damage, such as by treatment with antioxidant compounds, could be particularly effective at these stages of cancer development to delay or prevent malignant transformation. Head and neck, oral, esophageal, and certain skin cancers all appear to develop *p53* mutations relatively early (*42*, *96*) and all of these tissues probably have significant exposures to DNA-damaging agents during cancer development; such cancers might similarly benefit from such interventions that reduce further DNA damage. Alternatively, if a premalignant cancer cell retains wild-type p53, induction of apoptosis could retard the rate of progression to a more malignant state. The ability to screen body fluids such as sputum, urine, and stool (*97*) for genetic mutations in small numbers of cells will facilitate prevention strategies for a number of cancer types.

Restoration of checkpoint function in cells with abrogated checkpoints is another potential mechanism for minimizing cancer progression in these settings but is likely to be a technically more difficult task. Potential approaches to manipulation of p53 function include gene transfection techniques and biochemical manipulations that alter the conformation of the mutant p53 protein to restore wild-type function (*98*). Inactivation of the MDM2 protein, an endogenous inhibitor of p53 function (*29*, *99*), provides another potential target for elevating p53 function.

Knowledge of the role of cell cycle checkpoints in the cellular response to DNA damage suggests the existence of a class of environmental carcinogens that may have escaped detection (*100*). For example, environmental agents that abrogate the G_1-S checkpoint could dramatically enhance genomic instability in response to other DNA-damaging agents or even in response to intrinsic cellular "toxins" such as oxidative damage. Identification and elimination of environmental agents that act by inhibiting checkpoints or DNA repair may be an effective cancer prevention strategy.

Summary

Recent advances in our understanding of the cell cycle have revealed numerous regulatory processes that ensure the order of events in the cell cycle and integrate repair processes with cell cycle progression. Defects in these cell cycle controls can render the normal responses to damage ineffective and can lead to genomic instability and progression to malignancy. As our knowledge of these processes increases, we will be able to use molecular and cellular assays to assess the cell cycle controls missing in specific tumors. This characterization may dictate the choice and schedule of agents to be used in therapy. New compounds are likely to be developed that take advantage of the differences between cell cycle control in normal and cancer cells to maximize therapeutic effectiveness. Many of these new agents may be biological modifiers, rather than nonselective cytotoxic agents, that influence how cells respond to cytotoxic agents in terms of cell cycle perturbations and cell death pathways. For some cancers, the ultimate therapy—prevention strategies—may also be devised on the basis of this knowledge.

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