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# The cell cycle and cancer

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Recent insights in the fields of cell cycle regulation and cancer would each alone have provided prime examples of research at the "Frontiers of Science." However, some of the most revealing information about both topics has derived from the intersection of the two fields. The intent of this summary is to introduce the basics of the cell cycle, cancer, and their overlap, and then to describe the research from two laboratories that was presented in the session. A more comprehensive treatment of these subjects, beyond this description for a general audience, is contained in several reviews (1–5).

The process of replicating DNA and dividing a cell can be described as a series of coordinated events that compose a "cell division cycle," illustrated for mammalian cells in Fig. 1 (see legend for details). At least two types of cell cycle control mechanisms are recognized: a cascade of protein phosphorylations that relay a cell from one stage to the next and a set of checkpoints that monitor completion of critical events and delay progression to the next stage if necessary. The first type of control involves a highly regulated kinase family (2). Kinase activation generally requires association with a second subunit that is transiently expressed at the appropriate period of the cell cycle; the periodic "cyclin" subunit associates with its partner "cyclin-dependent kinase" (CDK) to create an active complex with unique substrate specificity. Regulatory phosphorylation and dephosphorylation fine-tune the activity of CDK–cyclin complexes, ensuring well-delineated transitions between cell cycle stages. In the future, additional molecular definition of the cell cycle may lead to a more intricate progression than indicated in Fig. 1.

A second type of cell cycle regulation, checkpoint control, is more supervisory. It is not an essential part of the cycle progression machinery. Cell cycle checkpoints sense flaws in critical events such as DNA replication and chromosome segregation (4). When checkpoints are activated, for example by underreplicated or damaged DNA, signals are relayed to the cell cycle-progression machinery. These signals cause a delay in cycle progression, until the danger of mutation has been averted. Because checkpoint function is not required in every cell cycle, the extent of checkpoint function is not as obvious as that of components integral to the process, such as CDKs.

Superficially, the connection between the cell cycle and cancer is obvious: cell cycle machinery controls cell proliferation, and cancer is a disease of inappropriate cell proliferation. Fundamentally, all cancers permit the existence of too many cells. However, this cell number excess is linked in a vicious cycle with a reduction in sensitivity to signals that normally tell a cell to adhere, differentiate, or die. This combination of altered properties increases the difficulty of deciphering which changes are primarily responsible for causing cancer.

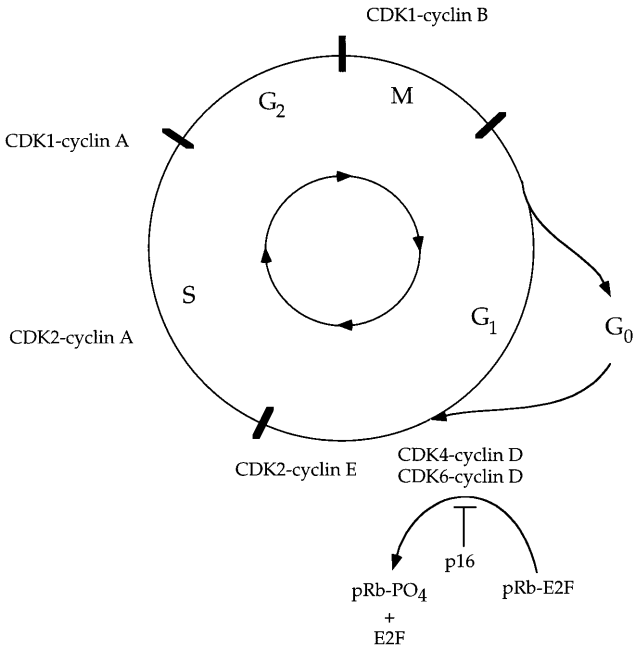


FIG. 1. A schematic representation of the mammalian cell cycle. In each cell division cycle, chromosomes are replicated once (DNA synthesis or S-phase) and segregated to create two genetically identical daughter cells (mitosis or M-phase). These events are spaced by intervals of growth and reorganization (gap phases $G_1$ and $G_2$). Cells can stop cycling after division, entering a state of quiescence ($G_0$). Commitment to traverse an entire cycle is made in late $G_1$. Progress through the cycle is accomplished in part by the regulated activity of numerous CDK–cyclin complexes, indicated here and described in the text.

The first genetic alterations shown to contribute to cancer development were gain-of-function mutations (6). These mutations define a set of "oncogenes" that are mutant versions of normal cellular "protooncogenes." The products of protooncogenes function in signal transduction pathways that promote cell proliferation. However, transformation by individual oncogenes can be redundant (mutation of one of several genes will lead to transformation) or can be cell type-specific (mutations will transform some cells but have no effect on others). This suggests that multiple, distinct pathways of genetic alteration lead to cancer, but that not all pathways have the same role in each cell type.

More recently, the significance of loss-of-function mutations in carcinogenesis has become increasingly apparent (7). Mutations in these so-called "tumor suppressor" genes were


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Abbreviation: CDK, cyclin-dependent kinase.

initially recognized to have a major role in inherited cancer susceptibility. Because inactivation of both copies of a tumor suppressor gene is required for loss of function, individuals heterozygous for mutations at the locus are phenotypically normal. Thus, unlike gain-of-function mutations, loss-of-function tumor suppressor mutations can be carried in the gene pool with no direct deleterious consequence. However, individuals heterozygous for tumor suppressor mutations are more likely to develop cancer, because only one mutational event is required to prevent synthesis of any functional gene product.

It now appears that tumor suppressor gene mutations are highly likely to promote, and may even be required for, a large number of spontaneous as well as hereditary forms of cancer (5). But what are the functions of tumor suppressor gene products in a normal cell? Although this is a topic for future research, there is suggestive evidence that several tumor suppressor genes encode proteins that negatively regulate cell cycle progression. Loss of function of the tumor suppressor gene product pRb, for example, would be predicted to liberate E2F transcriptional activators without requiring phosphorylation and thus bypass a normal negative regulation controlling entry into the cycle (Fig. 1). Loss of the tumor suppressor gene product p16 would have a similar consequence, liberating E2Fs by increasing pRb phosphorylation (Fig. 1). In addition, cell cycle progression can be halted at several points by the tumor suppressor gene product p53, activated in response to checkpoints sensing DNA and possibly also chromosome damage; loss of p53 would remove this brake to cycling (8).

By what molecular pathway does loss of cell cycle regulation in an organism lead to cancer? What genetic changes can cooperate to accomplish the cancer cell's escape from the normal balance of cell growth? Tyler Jacks described results from his laboratory that addressed these questions, using mice and cell lines derived from mice that have been engineered to lack individual tumor suppressor gene products. To create "knock-out" mice, embryonic stem cells that can later be introduced back into a developing animal are subject to targeted mutagenesis of the gene of interest. Cells with one mutant gene copy are injected into early embryos, and mice that use the injected cells to form germ-line tissue are selected for breeding. Some progeny will be entirely heterozygous for the mutant gene; these mice can then be bred to obtain homozygous mutant animals.

One important insight from the studies of mice lacking tumor suppressor genes is the dependence of balanced cell numbers on not only the regulation of cell proliferation but also on the regulation of cell death. In the past, cell death was regarded as an accidental failure of normal cell function. However, often the opposite is true: genetic studies of cell death indicate a requirement for active death signals and directed execution (for review of proteins involved in cell death see ref. 9). One collection of experiments illustrates the significance of combining genetic alterations that deregulate both cell proliferation and cell death (ref. 10; see also refs. 11 and 12). Inactivation of pRb during embryogenesis promotes inappropriate cell cycle activity. This follows from the role of pRb in negatively regulating entry into the cell cycle (Fig. 1). In contrast to expectations, however, the increased cell cycle activity in Rb null mice does not result in a net increase in cell number. This is due to a commensurate increase in cell death that specifically eliminates the abnormally cycling cells. This cell death is often dependent on the function of p53, as demonstrated from the analysis of RB/p53 double-mutant embryos.

The function of p53 in sentencing inappropriately growing cells to death has implications for cancer development and chemotherapy. Murine tumors with functional p53 respond to chemotherapy by promoting their own demise, but those lacking p53 typically do not (13). A balance between cell proliferation and death likely functions during development to create a finely patterned body map. This normal function of the cell death pathway and the potential for tipping the balance too much toward death in some degenerative diseases will be exciting future topics of investigation.

Clearly, the products of cell cycle regulatory genes are critical determinants of cancer progression. But precisely how do gene sequence alterations and missing regulatory components affect the functioning of the cell cycle machinery? Having in hand molecular details of the protein structures would address this question and would also suggest strategies for cancer therapy. Nikola Pavletich described research in his laboratory that has yielded high-resolution structures of p53 and of inactive and active states of CDK2. These structures were determined from the x-ray diffraction patterns of purified, crystallized proteins.

Although p53 may serve many roles in the cell, its best-characterized function is as a transcriptional activator. The residues of p53 that are frequently mutated in cancer cells are critical for DNA binding (14). A p53–DNA co-crystal structure revealed that these frequently mutated residues fold together into one region of the surface of the protein (15). Thus, cancer-promoting mutations that occur throughout the primary sequence of the protein are in fact clustered in one functional domain.

Recent studies have focused on the structural basis for regulation of the CDKs, using CDK2 as a model system (for review of CDK regulatory mechanisms see ref. 2). In mammalian cells, CDK2 functions in S-phase with cyclin A as a partner (Fig. 1). The association of cyclin A modifies the previously determined CDK2 structure (16) by reorienting a catalytically critical glutamic acid into the catalytic cleft and moving away the regulatory loop that can block access of a protein substrate to bound ATP (17). Cyclin A binding stimulates CDK2 activity, but phosphorylation of threonine-160 is required for full activation. The crystal structure of threonine-phosphorylated CDK2 complexed with cyclin A reveals conformational change in the substrate-binding site and also a strengthening of CDK2–cyclin A interaction (18).

Finally, one mechanism for the inactivation of the CDK2–cyclin A complex was examined: binding of the inhibitor p27 (19). Co-crystals of CDK2–cyclin A with the N-terminal inhibitory domain of p27 reveal that bound p27 physically blocks the active site, inserting itself into the catalytic cleft. Also, p27 association modifies the structure of the "roof" of the ATP-binding site and blocks a putative protein substrate docking region on cyclin A. With these structural modifications in mind, it may be possible to design small molecules that will have the same effect: blocking CDK activity, thus halting the cancer cell cycle in its tracks.


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