How Cells Cycle Toward Cancer

Until recently, the protein machinery of the cell cycle and the causes of cancer were thought to be separate topics. Now they appear to be merging

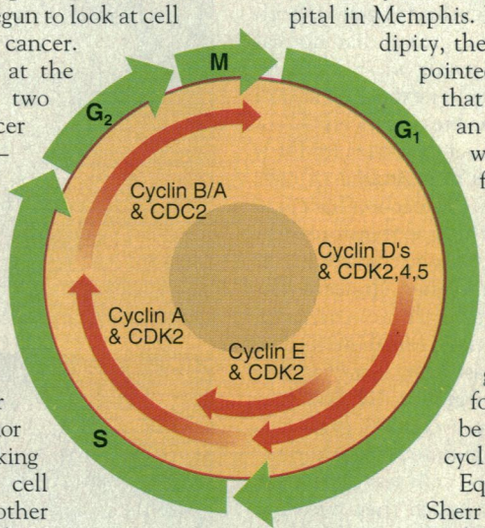
Over the past few years cell biologists have made remarkable progress in identifying the molecules that drive the cell cycle: the carefully choreographed series of events that culminates in cell division. In doing so they have not only provided a better understanding of one of the most fundamental of the cell's activities, they have also opened a new direction for research aimed at pinpointing the genetic changes that lead to cancer. The reason for this intriguing convergence is that accumulating evidence indicates that derangements in the cell cycle machinery may contribute to the uncontrolled cell growth characteristic of a tumor.

Some of these cell cycle derangements stem from outside influences. Many cancer-causing oncogenes, for example, turn out to encode components of the pathways through which growth factor signals feed into the cell cycle to stimulate cell division. And the tumor suppressor genes, which normally help keep cell growth in check, may also operate through the cell cycle. The recent demonstration that the protein encoded by the *p53* tumor suppressor gene apparently inhibits cell growth by turning on the production of a protein that blocks the cell cycle is a case in point (*Science*, 10 December 1993, p. 1645). "The basic message is that in every case where we have been looking at how growth controlling signals determine whether a cell proliferates, we have been able to make a connection between the growth controlling signals and cell cycle proteins," says molecular biologist Jim Roberts of the Fred Hutchinson Cancer Center in Seattle.

But the problem may go even deeper than that: Intrinsic defects in the cell cycle machinery itself may help cause cancer. And that comes as a surprise to many cancer researchers. "The presumption was that the heart of the [cell cycle] machinery wasn't going to be substantially altered in tumor cells, and that we can now say is categorically wrong," says David Beach of Cold Spring Harbor Laboratory, whose lab has been among the leaders in the cell cycle work.

Indeed, the gene encoding one component of the cell cycle machinery, a protein called cyclin D1, is apparently an oncogene itself and several others are oncogene candidates. Still other genes, which code for a group of newly discovered cell cycle inhibitors, including the one made in response to the p53 protein, have the potential to be tumor suppressors. All in all, the cell cycle genes are "an exciting new set of genes that could be targets for carcinogenesis," says Bernard Weinstein of Columbia University's College of Physicians and Surgeons, a longtime pioneer of carcinogenesis research who has himself recently begun to look at cell cycle derangements in cancer.

The new findings at the intersection of these two lines of research—cancer and the cell cycle—may have clinical implications, since the cell cycle components that have been implicated in cancer development might provide new targets for therapeutic strategies. It might be possible, for example, to inhibit tumor cell division by blocking the activation of the cell cycle by cyclin D1 or other cyclins, or by mimicking the effects of the inhibitors. In addition, it might be possible to use the information to determine a patient's prognosis, since there is evidence that the cell cycle derangements become worse as tumors progress to a more malignant state.



Spin control. The cyclins with their Cdks move cells through the cycle until they divide in M (mitosis) phase.

ILLUSTRATION: K. SUTLIFF

The best oncogene candidate

Of all the evidence that points to the direct involvement of cell cycle components in cancer, some of the best comes from studies of cyclin D1. This protein is one of a group of eight or so cyclins so far discovered in mammalian cells. Their name comes from the fact that their concentrations rise and fall in a regular pattern during the cell cycle, a pattern that enables them to do their critical job: turning on, at the appropriate moment, enzymes called cyclin-dependent kinases (Cdks), whose activity is needed to propel cells through the cell cycle. If the cyclins are overproduced in a cell or made at the wrong time, they would be expected to stimulate inappropriate cell division by keeping their partner kinases "on" when they should be turned off—a malfunction that could lead to cancer. That is just what appears to be happening with cyclin D1, as work begun in 1991 shows.

In that year, D cyclin genes were cloned by three independent groups, one led by Andrew Arnold of Harvard's Massachusetts General Hospital, another by Cold Spring Harbor's Beach, and the third by Charles Sherr of St. Jude Children's Research Hospital in Memphis. By a stroke of serendipity, the Arnold team's work pointed to the possibility that cyclin D1 might be an oncogene. They were looking for a defective gene, mapped to band q13 of chromosome 11, which was thought to be the cause of benign tumors of the parathyroid gland. The gene, when they found it, turned out to be none other than the cyclin D1 gene.

Equally intriguing, the Sherr and Beach groups' work showed that the D cyclins are active at a particularly critical time in the cell cycle: during the G1 phase when cells grow and decide whether to begin replicating their DNA in preparation for cell division. "The D cyclins act as growth factor sensors to help a cell make a decision to divide," Sherr says. "But once cells commit to [DNA] synthesis, they've committed to the rest of the cell cycle." At that point cell division can proceed without any stimulation by growth factors. The discovery was suggestive in relation to cancer, since one of the hallmarks of cancer is cell division without the need for external stimulation.

That wasn't the only evidence implicating the cyclin D1 gene in cancer. Band q13 of chromosome 11 harbors abnormalities linked to several cancers in addition to benign parathyroid tumors. As one example, an oncogene called *bcl1*, which may help cause B cell lymphoma, had been mapped to the region. The discovery of the cyclin D1 gene at the same site immediately raised suspicions that it might be the *bcl1* gene, which had not been cloned at the time. Since then, that suspicion has been confirmed by several groups, including Arnold's. "There's virtually no doubt now that the cyclin D1 gene is the *bcl1* oncogene," he says.

Overexpression of the gene for cyclin D1 may contribute to more common cancers, including those of the breast and esophagus, in addition to the B cell lymphoma. These other cancers may also show abnormalities at 11q13, particularly amplifications in which the chromosomal region is repeated several times. That somewhat complicates matters since the amplified region contains other genes, including two known to have oncogenic activity, whose overactivity might lead to abnormal growth. The bulk of the evidence, however, points to the cyclin D1 gene, since the other two oncogenes do not appear to be overexpressed, even when they are amplified. In contrast, Weinstein and colleagues find that the cyclin D1 gene is both amplified and producing greater than normal amounts of protein in nearly one-third of the 50 esophageal cancers they examined as well as in about 15% of the breast cancers.

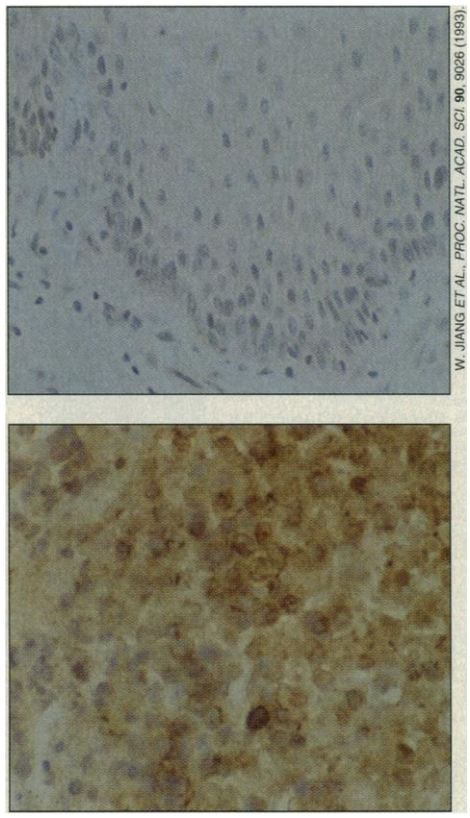
Further evidence that excess cyclin D1 is causing cancer comes from studies of cells growing in lab culture. Researchers have had trouble showing that they can transform normal cells into cancer cells by transferring in active copies of cyclin genes. (Indeed, that was one reason intrinsic cell cycle derangements weren't thought to contribute to cancer development.) Last year, however, the Sherr and Weinstein groups did find that overexpression of the cyclin D1 gene perturbs the cell cycle in cultured cells, shortening G1 and making the cells less dependent on growth factors, although they were not visibly transformed. Weinstein and his colleagues also found that their cells produce tumors when injected into nude mice, although less effectively than cells transformed with conventional oncogenes.

And a group including Philip Hinds of Harvard Medical School, Robert Weinberg of the Whitehead Institute for Medical Research in Cambridge, and Arnold has now shown that in some circumstances the cyclin D1 gene can cooperate with the *ras* oncogene in transforming cells—a finding consistent with the large body of evidence showing that several gene changes are required to produce most cancers. "The effects of these [cyclin] genes on the proliferative state are subtle," concludes Sherr, "but one can think that they might contribute in a multi-step process to disease."

And cell cycle derangements may do more to push cells into cancer than just disposing them to increased growth. Sherr's group has found that cyclins D2 and D3 block cell differentiation—another hallmark of cancer cells. What's more, releasing the blocks on cell growth can allow cells to accumulate mutations and chromosomal abnormalities, including those that foster cancer development. "The most important new way of looking at cancer for the field as a whole is recognizing the importance of increased cellular evolution in cancer cells," says Leland Hartwell of the University of Washington in Seattle. Indeed, *p53*'s normal function apparently is to sense DNA damage and stop cells from dividing until it's repaired.

Other cell cycle derangements

While cyclin D1 has received the lion's share of attention, it is hardly the only cyclin whose expression is deranged in cancer cells. Khandan Keyomarsi and Arthur Pardee of the Dana-Farber Cancer Institute found that the gene for cyclin E, which also



Too much of a good thing? Staining shows much more cyclin D1 in esophageal cancer cells (bottom) than in normal epithelium.

becomes active during the G1 phase of the cycle, is overexpressed in cultured breast cancer cell lines and in primary breast tumors. Furthermore, whereas normal cells contain only one detectable species of cyclin E protein, cancer cells contain three varieties with different molecular weights. "Cyclin E is a very important player," says Pardee. "We see dramatic changes in cyclin E in breast cancer cells."

Keyomarsi, Pardee, and their colleagues find that the concentrations of all three forms of cyclin E increase as breast tumors progress toward greater severity. That correlation, they say, suggests that cyclin E might be a new and valuable marker of a patient's likely prognosis, since it can indicate the stage of her tumor at the time of diagnosis. And the marker may not be limited to breast tumors: The Dana-Farber workers also saw increased cyclin E production in a wide variety of other cancers, including such common ones as lung, colon, and ovarian cancers.

Cyclin A may also be involved in oncogenesis, Roberts says. He notes that it, like cyclins D and E, is important for completion of G1 and passage into the DNA-synthesizing stage of the cell cycle. In addition, his group has shown that overexpression of the cyclin A gene leads to another classic feature of cancer cells: the ability to grow without being anchored to a surface.

The interest in the cyclins notwithstanding, Sherr points out that cancer researchers shouldn't overlook other elements of the cell cycle machinery, including the cyclins' partners, the kinases, as oncogene possibilities. Among the half dozen kinases that have been identified, the one called Cdk4 is a particularly good candidate, he says. For one thing, the Cdk4 gene, which maps to a region on chromosome 12, is amplified in some cancers. For another, Mark Ewen, David Livingston, and their colleagues at Dana-Farber, have recently shown that transforming growth factor β (TGF-β), which, despite its name, is a major *inhibitor* of growth in most cell types, acts by blocking Cdk4 synthesis. Moreover, cells that have been altered so that they produce Cdk4 all the time cannot be inhibited by TGF-β. Since many cancer cells lose their responsiveness to TGF-β's growth-suppressing effects, these results raise the possibility that Cdk4 production may contribute to the failure of growth controls in cancer cells. If so, the enzyme would be a promising new target for anticancer drugs.

Tumor suppressor candidates, too

If the cyclins and Cdks contribute to cancer, they would presumably act positively, like the oncogenes. Researchers are speculating, however, that the newly discovered cell cycle inhibitors might be more like the negatively acting tumor suppressors whose loss or inactivation leads to cancer. Cancer researcher Ed Harlow of Massachusetts General Hospital describes the discovery of these inhibitors as "a major breakthrough. There's a level of control for mammalian G1 that's not used" by nonmammalian species.

Currently, there are three such inhibitors. One, whose discovery was announced at the end of last year by four groups, is made in response to p53 and apparently mediates its growth suppressive effects by blocking the activity of Cdk2 and other Cdks. The second cell cycle inhibitor, discovered by Beach and his colleagues, is more specific in its action, apparently blocking only Cdk4. The third, discovered by a group including Joan Massague of Memorial Sloan-Kettering Cancer Center in New York City, as well as Roberts and Sherr, helps to mediate TGF-β's inhibitory effects and

also the growth inhibition brought about when cells come into contact with another.

Since, as cancer gene expert Tony Hunter of the Salk Institute puts it, "any negative regulator of the cell cycle is a potential target for inactivation"—and therefore cancer development—the discovery of these inhibitors raises the possibility that they might themselves be tumor suppressors. Mutations in these genes might, for example, contribute to the development of the 50% of human cancers in which mutations in *p53* itself do not occur, although this has yet to be demonstrated.

Researchers clearly still have a lot to do to pin down the role of the cell cycle components in causing cancer. One of the biggest gaps in their knowledge concerns the identity of the targets of the cell cycle kinases, information they want to help understand exactly what turns on DNA synthesis and moves cells through the cycle. So far one such target has been identified, and it's an important one from the point of view of understanding cancer: the protein product of the retinoblastoma (*Rb*) gene, which is another tumor suppressor.

A few years ago, researchers in several labs found that *Rb* suppresses cell growth by binding to a transcription factor and preventing it from doing its normal job of turning on gene expression. That block is relieved when the cell cycle kinases are activated and add phosphates to *Rb*, causing it to release the transcription factor (*Science*, 14 June 1991, p. 1492). More recently, several groups have evidence that the D and E cyclins and their associated kinases are particularly important in overriding *Rb*'s inhibitory effects, and moving cells from G1 into DNA synthesis and cell division. But, predicts Harlow, whose own research includes *Rb*, "*Rb* is not going to be the only one [cell cycle target]."

Indeed, there are now numerous links between the cell cycle and growth factors on one hand and tumor suppressors on the other. Add the idea that intrinsic defects in the operation of the cell cycle can also lead to cancer, and it's clear that cell cycle research will be going around at a high rate of speed for a long time to come.

–Jean Marx

Additional Reading

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TECHNOLOGY

Researchers Try to Build Time Machines for Microwaves

"Ginger Rogers did everything Fred Astaire did," say feminists. "She just did it backward and in high heels." Physicists trying to "time-reverse" beams of microwaves have a vivid appreciation of how difficult it can be to do things backward. These researchers are trying to design reflectors that undo distortion in a microwave signal by sending it back to its source exactly as it was originally transmitted. A simple mirror won't suffice; all it can do is change a signal's direction. Instead, the reflecting medium has to shape the outgoing beam so that it precisely reverses every motion of the incoming beam. If the original beam came from the left, for example, the outgoing beam has to angle to the left; if the original beam fanned out, the outgoing beam has to converge; if the original beam got distorted, the outgoing beam has to be distorted in reverse—so that it will lose the distortion on the return trip.

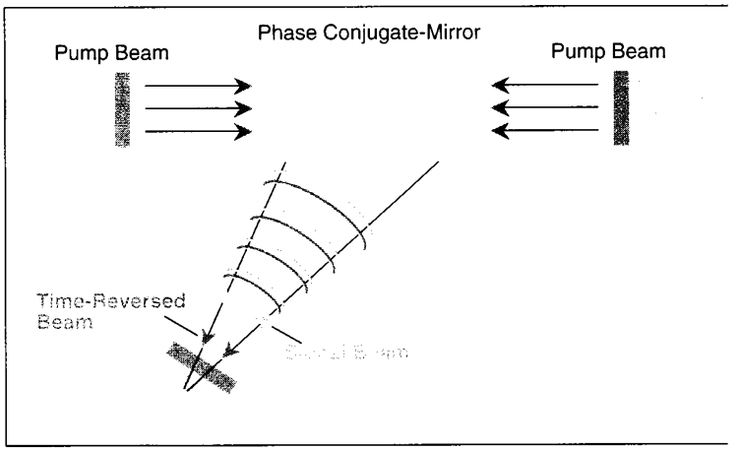
Just as Ginger Rogers might have turned an ankle or toppled a lamp during a grueling routine, physicists trying to master this feat have gotten used to setbacks. But in the last couple of years, several of them of them have taken a crucial step: They've developed exotic materials that can serve as wave-reversing mediums for microwaves —plasmas, stacks of tiny polysilicon pendulums, and liquid suspensions of graphite fibers. None of these materials so far produces a time-reversed signal strong enough for practical applications. But Harold Fetterman of the University of California, Los Angeles, for one, thinks he and his colleagues are on the track of what he calls "a definitive experiment in opening up the technology."

Motivation for this work comes from earlier successes with visible light. Phase-conjugate mirrors (PCMs), as they are called, have found dozens of important applications in the visible spectrum, from undoing distortion in laser beams to reconstructing images that get scrambled when transmitted through long stretches of optical fiber. For phase conjugation of microwaves, the potential applications are, if anything, more numerous. Equipped with a PCM, a satellite could beam data unerringly to a ground station; a radar system could turn a weak reflection from a distant object into a powerful, directed probe.

These goals have been tough to achieve for microwaves because phase conjugation requires each incoming signal to, in effect, reshape the "mirror." The PCM has to record the contours and phases of the incoming wavefronts by temporarily changing its own characteristics, such as its index of refraction. The outgoing beam will then pick up this "phase information," acquiring exactly the same contours and phases—but in reverse temporal order—as it passes through the altered regions of the PCM.

In a common scheme for time-reversing an optical signal, called four-wave mixing, other beams help to record the phase information, then write it into the outgoing beam. The incoming beam (wave *1*) interferes with a "pump" beam (wave *2*), creating a pattern of light and dark regions within the PCM that encodes information about the incoming beam. This interference pattern reshapes the optical properties of the PCM. When a second pump beam (wave *3*) travels through the medium in the opposite direction from the first, some of it gets imprinted with a record of the incoming beam's characteristics and leaves the medium as the phase-conjugated wave (*4*). Because the pump beams can be far more intense than the incoming beam, four-wave mixing yields a valuable bonus: The PCM can exhibit "gain," amplifying the original signal to generate a far stronger time-reversed beam.

SOURCES: *SCIENTIFIC AMERICAN*; DOMIER ILLUSTRATION: C. FABER SMITH



Return to sender. In four-wave mixing, incoming microwaves interfere with a pump beam *(right)* in a special medium, perturbing it. The perturbations scatter a second pump beam, time-reversing the signal.