CELL BIOLOGY

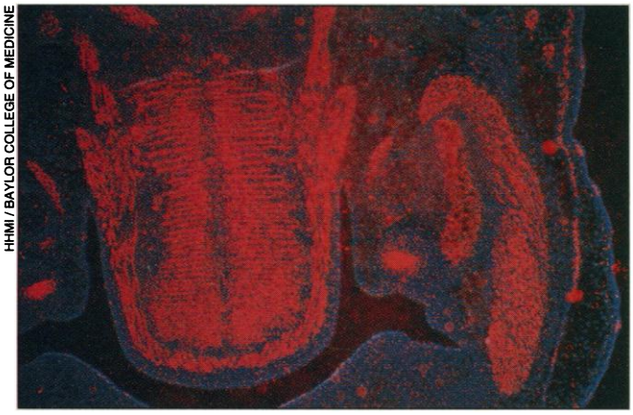
Cell Cycle Inhibitors May Help Brake Growth as Cells Develop

For a normal developing cell, the decision to commit to becoming a particular type of specialized cell carries a heavy price. The cell must give up the prospect of achieving immortality through its progeny, withdraw from the cell cycle, and stop dividing. That may sound dire, but it is necessary for the well-being of the organism. "Once the developmental program is executed, it's very important that you shut off the cell cycle and keep cells from dividing," notes geneticist Steve Elledge of Baylor College of Medicine in Houston. Even one cell dividing when it shouldn't can lead to cancer, and if the problem were widespread, normal formation of tissues and organs would be disrupted. Just what causes cells to stop dividing when they differentiate into their specialized cell types, however, has been a mystery.

Now a burst of new studies is offering some clues.Three papers in this issue, one from a group including Elledge and Baylor biochemist Wade Harper and two from a team led by Andrew Lassar of Harvard Medical School, suggest that one reason muscle cells stop dividing as they differentiate is that they produce a protein, p21, that inhibits a key enzyme needed for the cell cycle (see pp. 1018, 1022, and 1024). What's more, the involvement of cell cycle inhibitors in stopping cell growth during differentiation may not be limited to muscle cells, because researchers have made similar findings, some not yet published, in other cell types.

Stave Kohtz of Mount Sinai School of Medicine in New York City, who is also studying links between the cell cycle and differentiation, calls the new results "fascinating." And if they are borne out by further work, there could be a larger payoff, says Kohtz, because understanding how the cell cycle is coordinated with differentiation is "fundamental to understanding the molecular basis" of such basic developmental problems as the formation of structures in the developing embryo.

The work also provides a good example of how previously diverse fields can merge, because Lassar and Elledge came to the current subject from different directions: Lassar from a longtime interest in muscle differentiation and Elledge from his specialty, cell cycle regulation. An early clue that pointed muscle researcher Lassar toward the cell cycle came 2 years ago when Bernardo Nadal-Ginard's group, also at Harvard Medical School, and Lassar's own group independently showed that the growth-inhibiting activity of the protein produced by the retinoblastoma (Rb) tumor suppressor gene is needed to keep differentiated muscle cells from dividing. That finding provided a link to the cell cycle, because researchers had previously shown that the activity of the Rb protein is controlled by key cell cycle enzymes, the cyclin-dependent kinases (cdks). The cdks regulate the activity of other proteins by adding phosphate groups to them, and cdk phosphorylation of the Rb protein effectively puts it out of commission, allowing the cell cycle to proceed. But in the case of differentiating muscle cells, the question was not what turns Rb off. The question of interest was how is Rb turned on so that it stays on and inhibits cell division in specialized cells?



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Oral arguments. Areas where the *p21* gene is expressed in tongue and masseter muscles are shown in a coronal section through oral cavity of a wild-type mouse embryo.

Beginning just over a year ago, several groups, including that of Elledge and Harper, found a good place to start looking for the answer. They identified several proteins that inhibit the cdks (*Science*, 10 December 1993, p. 1644). As Lassar puts it, "It didn't take a rocket scientist to realize" that production of such proteins, by inhibiting the cdks, could allow the Rb protein to do its job of taking differentiating muscle cells out of the cell cycle.

Early results support that hypothesis. In the paper that appears on page 1018, Lassar and his colleagues report that as muscle cells differentiate in lab culture they induce synthesis of one of the inhibitors, p21. "The nice aspect of this correlation is that it provides a mechanistically simple way to couple muscle differentiation and cell cycle arrest," Lassar says.

The new work goes beyond that finding to suggest how p21 synthesis might be turned on in differentiating muscle cells. Over the past few years, researchers have learned that as muscle cells begin to differentiate, they produce a family of transcription factors, called the myogenic basic helix-loop-helix (bHLH) proteins. These factors trigger the activity of genes that make characteristic muscle proteins, such as myosin, a component of muscle's contractile apparatus. Both the Lassar group and the Harper-Elledge team, which includes developmental biologist Gregor Eichele of Baylor and muscle development specialist Eric Olson, who is at M. D. Anderson Cancer Center in Houston, have shown that in cultured cells one of these bHLH proteins, MyoD, can turn the *p21* gene on. This effect, incidentally, does not require the activity of the p53 tumor suppressor protein, which had been shown in previous work to activate *p21* expression in response to DNA damage.

Equally interesting, Lassar suggests that p21 may play a dual role, actively fostering muscle cell differentiation in addition to inhibiting cell division. The researchers found that expressing the genes for p21 and another cell cycle inhibitor, p16, in proliferating immature muscle cells stimulates production of muscle-specific proteins. Intriguingly, p21 and p16 may produce their dual effects via exactly the same mechanism—inhibiting cdk action. As their name indicates, the activity of the cyclin-dependent kinases depends on cooperation by partner proteins called cyclins. The Lassar group in the current studies (along with Kohtz and colleagues in previous work) find that the activity of the cyclin designated D1 inhibits MyoD's ability to stimulate expression of muscle-specific genes. As Lassar puts it, "you're getting two things for the price of one with these cdk inhibitors."

The idea that p21 might couple differentiation and inhibition of the cell cycle was given further plausibility by the work of the Elledge-Harper team. While the Harvard group's work was all done in cultured cells, the Texas workers examined expression of cell cycle inhibitors in developing mouse embryos. They found that production of p21 is turned on in muscle cells of the developing embryo just when those cells begin undergoing terminal differentiation. "Our paper shows the inhibitors are expressed at the right time in the embryo to be part of the regulatory mechanism," says Olson.

And that regulatory pattern may be found in more than just muscle cells. The Texas

team has found that the *p21* gene is turned on during terminal differentiation of several other cell types, including cartilage, skin, and the lining of the nasal passages. Other researchers including Paolo Dotto of Harvard Medical School, working with skin cells, and Bert Vogelstein of Johns Hopkins University School of Medicine, working with cells of the intestinal lining, are finding correlations between p21 expression and commitment of cells to their mature fates.

In spite of this accumulating evidence, the link between p21 and terminal differentiation is not airtight. For one thing, the results from embryos differ in a significant way from the cultured cell data: The myogenic bHLH protein MyoD turned on p21 expression in the cultured cells, but it is not necessary for activation of the gene, because p21 production occurred even in embryos in which the MyoD gene had been inactivated. Elledge says this doesn't necessarily mean the myogenic bHLH proteins have no role in turning on p21. He notes that another of these proteins, Myf-5, has the same range of activities as MyoD and might be doing the job in MyoD's absence.

But perhaps the biggest caveat is that the researchers are still working at the level of correlations. Simply finding that p21 is turned on as cells differentiate doesn't necessarily mean it plays the postulated role in coupling differentiation to growth cessation. Although molecular biologist Robert Weinberg of the Whitehead Institute for Biomedical Research says he finds the correlations "interesting," he nevertheless cautions that "correlations are no longer as exciting as they were." Weinberg is referring to the fact that there are now more direct ways to test whether a protein has a particular function. Its gene can be knocked out, for example, or specific antibodies can be used to block the protein's activity.

The researchers doing the work are the first to concede that these more definitive tests need to be done. As Lassar says, "The proof of the pudding for the speculated role [of p21] awaits the functional inactivation of the gene product." But even if p21 itself strikes out as a link between the cell cycle and differentiation, other cell cycle inhibitors are waiting their turn at bat. Kohtz says, for example, that his work points to one of these, dubbed p27, as possibly important in muscle. In fact, there's already preliminary evidence that several cell cycle inhibitors besides p21 may be involved.

Indeed, Elledge says, efforts to pin down the role of the cell cycle inhibitors in differentiation is just getting off the ground. But exploring just when and where they are turned on in the embryo may eventually give researchers a more precise view of how organisms develop.

–Jean Marx

MATHEMATICS

A Visit to Asymptopia Yields Insight Into Set Structures

With computer circuitry and telecommunication networks growing in complexity by leaps and bounds, researchers who analyze the fundamental mathematical features of these finite but increasingly large systems often must scramble to keep pace. But Jeong-Hon Kim of AT&T Bell Labs in Murray Hill, New Jersey, decided to beat technology to the punch. Kim was trying to determine the size at which networklike systems inevitably develop certain specific structures, but he gave up examining systems of finite size because they became too complex. Instead, he took a new approach. He looked for *asymptotic* results: theorems that set bounds on the behavior of large systems rather than describing it exactly. Such theorems still apply—indeed, they grow more precise—as the systems grow infinitely large.

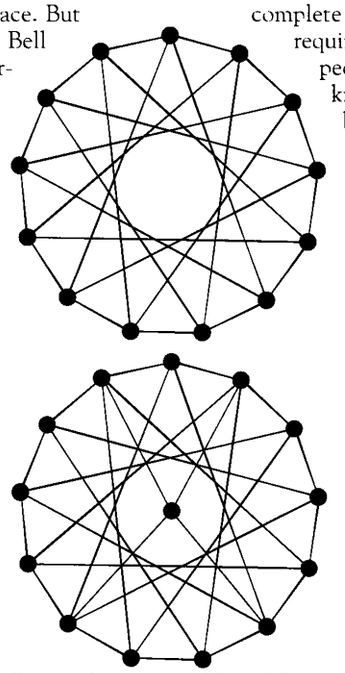

ILLUSTRATION C. FABER SMITH

Sets and structure. A "graph" of 13 connected points can be drawn to have no triangles and no set of five points without one link in common *(top)*, but when the graph grows to 14 points, one constraint or the other breaks down *(red)*.

Kim's effort yielded a major step forward in Ramsey theory, a branch of mathematics concerned with the unavoidability of "accidental" structures in large systems. His success is also a vindication of a strategy that a growing number of researchers are adopting, as Joel Spencer of the Courant Institute of Mathematical Sciences described last month in San Francisco at the joint meetings of the American Mathematical Society and the Mathematical Association of America. Searching for asymptotic rather than exact solutions has a key advantage, Spencer argued in a talk called "Adventures in Asymptopia": It makes available the arsenal of tools from calculus and differential equations, which don't readily apply to finite-sized systems. Speaking with the enthusiasm of a mathematical travel agent, Spencer described Asymptopia as "a magical place where all the problems that you have with discrete calculations just melt away!"

What Kim found in Asymptopia was the solution to a vexing problem in Ramsey theory that is sometimes described as the party problem. Suppose you want to throw a party and are deciding on a guest list. On the one hand, you want people to mix, so you don't want to wind up with any "triangles" consisting of three people each of whom already knows the other two. On the other hand, you don't want to wind up with any large groups of complete strangers, so you decide to require that among any, say, five people, at least two should know each other. Satisfying both constraints gets harder as the guest list gets longer, but just how large can the party get before the constraints break down? And how does the possible size of the party grow as you change the group size from five to, say, 10,000?

Mathematicians usually frame the problem in terms of graphs, which consist of points connected by line segments, or edges. Ramsey's theorem, which dates back to the 1920s, implies that for any number k (say $k = 5$), every graph with "sufficiently many" points either contains at least one "triangle"—that is, three points each connected to the other two—or else it contains a set of k "independent" points, none of them connected to each other. More generally, Ramsey theory says that almost any pattern can be found in a system with sufficiently many parts—a fact that undoubtedly helped early stargazers see constellations and is a bane of network designers, who can be blindsided by unexpected connection patterns.

The sticking point is the phrase "sufficiently many." Some graph theorists have focused on looking for exact values for the function they dub $R(3,k)$—the smallest size at which graphs are guaranteed to contain either a triangle or an independent set of size k. They have succeeded in finding values up to $k = 9$. But there the search has stalled as the proofs became increasingly intricate. So other graph theorists have concentrated their efforts on the asymptotics of $R(3,k)$—looking for formulas that, while not precise, give bounds that hold no matter how large k is.

By the early 1980s, graph theorists had succeeded in boxing the values of $R(3,k)$ between multiples of $k^2/(\log k)^2$ and $k^2/\log k$.