stem a geographic brain drain that shrinks the country's overall R&D capacity, says Gary Strobel, a microbiologist at Montana State University in Bozeman. "Most universities in rural states are better positioned to do work in biotechnology than in any other field because of their roots as land-grant agricultural schools," he says. "But the problem is that most of our young people get trained and go elsewhere for jobs because there aren't enough opportunities at home."

NSF officials, who point out that geographic diversity could be one of several factors in making an award, say barring the top universities from a competition is a bad idea. "We'd never do it," says Mary Clutter, head of NSF's biology directorate. "It would be sheer folly to exclude the best universities in the country."

A better alternative to earmarks, say some scientists and policy-makers, would be for Congress to provide funds aimed specifically at improving the research capacity of so-called "have-not" institutions and regions, and then use peer review to select individual award winners. NSF began such a program almost 20 years ago, called the Experimental Program to Stimulate Competition in Research (EPSCoR), and it has grown to a $100 million effort at eight agencies. "You might call EPSCoR an earmark, but it's not damaging to the system," says Erich Bloch, a former NSF director now at the Council on Competitiveness in Washington, D.C.

Such programs are unlikely to dampen the taste for scientific pork, however, and Maze believes researchers must learn to live with the practice. The rush to adorn the transportation bill with earmarks, he believes, "clearly demonstrated the power of winning university sponsorship through political muscle rather than through superior intellectual resources." **–DAVID MALAKOFF**

With reporting by Andrew Lawler, Eliot Marshall, and Jeffrey Mervis.

CELL BIOLOGY

How a Growth Control Path Takes a Wrong Turn to Cancer

As researchers work out how the Wnt pathway controls growth and development, they are getting a better grasp on the causes of cancer

Biologists these days often find themselves exploring isolated corners of the cell's molecular labyrinths. But every so often, the trails they have been following converge unexpectedly, and a unified picture emerges of a previously mysterious cell function. Recently, researchers studying one of the cell's key developmental and growth regulatory pathways—called the Wnt pathway after the protein that sets it in motion—have had that happy experience.

Over the past year or two, a confluence of evidence from molecular and cell biology as well as from research on development, cancer, and the immune system is providing a good look at how the pathway conveys signals all the way from the cell surface, where the Wnt protein binds to its receptor, to at least one gene in the nucleus. Out of this confluence is coming a better understanding not just of embryonic development in species ranging from fruit flies to humans but also of cancer.

Wnt pathway awry. Excessive Wnt signaling, caused by a defective *axin* gene, produces two-headed frog embryos (*left*). A normal embryo is at top.

CREDIT: ZENG *ET AL. CELL* **90**, 181 (1997)

Because activation of the Wnt pathway stimulates cell growth, researchers had long suspected that too much Wnt signaling could cause problems. The new work bears out those suspicions, showing how damage to a well-known tumor suppressor gene could turn on an equally prominent oncogene via the Wnt pathway and lead to cancer.

The tumor suppressor is the adenomatous polyposis coli gene (*APC*), which is lost or inactivated in some 85% of colorectal cancers. And as described on page 1509, the oncogene is the c-*MYC* gene. Although researchers linked inappropriate c-*MYC* activation to Burkitt's lymphoma and lung, colon, and other cancers 20 years ago, they hadn't been able to figure out exactly what causes c-*MYC* expression to go awry in most cases. But cancer geneticists Tong-Chuan He, Bert Vogelstein, Kenneth Kinzler, and their colleagues at Johns Hopkins University School of Medicine in Baltimore, Maryland, now report that they have identified one of the genes turned on by Wnt signals—and it's none other than c-*MYC*.

Normally, it seems, *APC* instructs the Wnt pathway to keep c-*MYC* expression in check until the right signal comes along, say, to stimulate the cell growth needed for embryonic development. But if *APC* is missing or inactive, c-*MYC* will be active all the time, causing tumor growth. "This paper is a major, unexpected contribution to our understanding of not only normal c-*MYC* control but [also] how the loss of a tumor suppressor can result in abnormal activation of c-*MYC*," comments Kenneth Marku, a molecular biologist at the State University of New York (SUNY), Stony Brook.

As they learn more about the Wnt pathway's involvement in cancer, researchers are also becoming more optimistic that they can put their results to work developing new anticancer drugs that act by blocking c-*MYC* activation. "We have a pretty good shot at doing something about colon cancer, now that we know a lot about this pathway," says Paul Polakis, a biochemist at Onyx Pharmaceuticals Inc. in Richmond, California. The potential benefits may not be limited to colon cancer, because other recent work suggests that Wnt pathway malfunctioning may contribute to the development of additional cancers, including the dangerous skin cancer melanoma and cancers of the prostate, liver, and possibly the breast.

Following the Wnt trail

Cancer researchers originally discovered the gene for Wnt, a protein that conveys growth and developmental signals between cells, 16 years ago in mouse mammary tumors. At the time, the gene was called *Int-1* because it became activated when the mouse mammary tumor virus inserted—or integrated—next to it in the genome. This abnormal activation led to tumors in the mice, marking *Int-1* as an oncogene. But a 1987 discovery showed that the gene has a role in normal embryonic development as well: It is the mouse version of *wingless* (*wng*), a developmental control gene first found in the fruit fly. Since then, much evidence has shown that *Int-1* (which was subsequently rechristened *Wnt-1*, a melding of *wingless* and *Int*) and its relatives control such aspects of development as the

formation of the central nervous system.

While the mouse mammary tumor link suggested that *Wnt-1* might also be an oncogene in humans, no evidence has been found for that. Beginning in the 1980s, however, evidence began building that the pathway that conveys developmental and other signals from *Wnt-1* to the nucleus could have its own role in cancer.

One clue came in the 1980s from Walter Birchmeier, a cell biologist at the Max Delbrück Center for Molecular Medicine in Berlin, Germany. At the time, his group was studying E-cadherin, a protein that projects out of the cell membrane and provides the "glue" that helps cells stick to one another. As a way of finding out more about E-cadherin function, Birchmeier began looking for molecules that interact with it in the cell. One of the proteins his search turned up was β-catenin. The fruit fly geneticists had already identified this protein as a component of the Wnt pathway by showing that adding β-catenin to insects with Wnt mutations restores proper development.

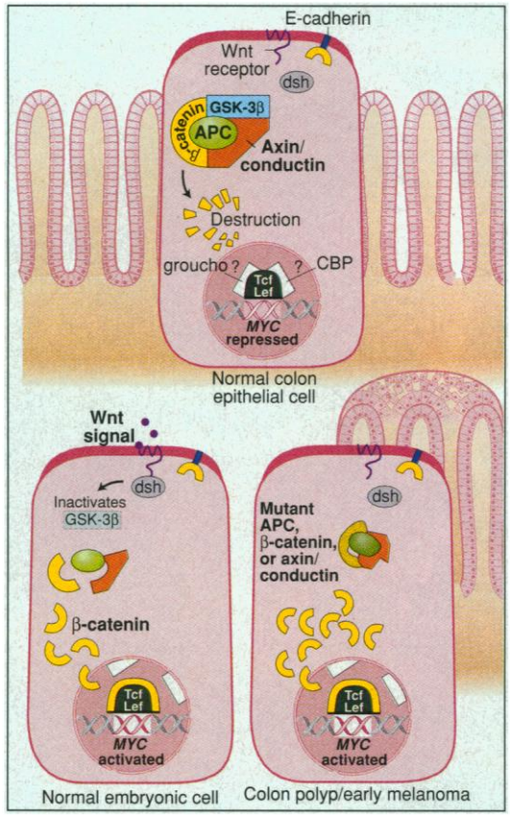
Soon Birchmeier's group had expanded its initial study of cell adhesion and was screening for additional components of the Wnt pathway by looking for molecules that interact with β-catenin. They found several. One, Birchmeier and Jürgens Behrens reported in 1996, turned out to be a member of the Lef/Tcf family of transcription factors, which regulate gene expression in certain immune cells. Hans Clevers, an immunologist at the University of Utrecht in the Netherlands, made a similar discovery at about the same time.

While everyone expected that Wnt signals would have to alter gene expression in order to play a role in development and cancer, no one had known how until then. "That was a major contribution in figuring out how the Wnt signal [worked] in the nucleus," says Polakis. Researchers could find no role of E-cadherin itself in Wnt signaling, however, since it normally holds onto its β-catenin. The β-catenin that makes it to the nucleus and interacts with Lef/Tcf comes from a different cellular pool.

Meanwhile, another discovery had also helped draw researchers' attention to the Wnt pathway. In 1993, the Polakis and Vogelstein groups both showed that the tumor suppressor gene *APC* was likely to be part of the pathway. The truncated, inactive form of the APC protein seen in most colon tumor cells, they found, resulted in β-catenin accumulation in the nucleus, where it might turn on genes. The normal form of APC, in contrast, prevented that accumulation. The result provided a possible explanation of why APC loss leads to cancer: by turning on the Wnt pathway—and the expression of certain genes—even under conditions when it would normally be shut down.

Indeed, subsequent experiments confirmed this scenario. Clevers and the Kinzler-Vogelstein team have shown that in cells lacking a working APC protein, β-catenin accumulates in the nucleus, where it gets attached to Tcf-4, the Lef/Tcf transcription factor active in gut tissue, and presumably stimulates the tumor cell growth. In about 10% of colon cancer cells, the Kinzler-Vogelstein team found, mutations in β-catenin itself have the same effect. "In a sense, you can't get colon cancer unless you disrupt this pathway," says molecular biologist Jan Kitajewski at Columbia University in New York City.

And not just colon cancer. Polakis and his colleagues have evidence that the Wnt pathway is disrupted in about one-third of melanomas, and Christine Perret's group at the University of Paris has recently found mutations in the β-catenin gene in mouse and human liver tumors. Some prostate and breast cancers may also arise from faulty Wnt-pathway components.



Signaling the nucleus. In normal colon cells (top), β-catenin is either held by E-cadherin or destroyed, preventing it from reaching the nucleus and relieving groucho and CBP's repression of c-*MYC*'s growth signals. But Wnt signals (bottom left) or mutations that block formation of the complex that targets β-catenin for destruction (bottom right) allow β-catenin buildup, c-*MYC* activation and thus cell proliferation.

ILLUSTRATION: K. SUTLIFF

Checking Wnt's signal

Now researchers are learning just how APC and other components of the Wnt pathway work together to transmit or silence Wnt's growth-stimulating signal. Researchers including Randall Moon and David Kimelman at the University of Washington School of Medicine in Seattle have shown that unlike tumor cells, nonproliferating cells have very little free β-catenin in the absence of Wnt signaling. Much of the β-catenin is tethered to cadherin molecules that protrude from the cell membrane. The rest is bound up in what several research teams have shown is a multiprotein complex with APC and two other proteins: a kinase enzyme called GSK-3β that tags β-catenin for degradation by adding phosphate groups to it and a protein newly discovered this year that is known either as axin or conductin, depending on the cell type.

Birchmeier and Behrens found that conductin apparently assembles this complex by linking the components together. This allows β-catenin phosphorylation and degradation to proceed, keeping the Wnt pathway in check. But as shown by the combined efforts of many labs, when Wnt binds to its receptor, that signal is relayed to a protein called dishevelled and from there to GSK-3β. As a result, β-catenin degradation is blocked and it can travel to the nucleus and interact with transcription factors, thereby regulating genes.

Damage to any part of this complex—not just APC—can lead to abnormal signaling. Developmental biologist Frank Costantini's team at Columbia University showed, for example, that mice with a mutated *axin* gene grow a second head and neural tube. Without *axin*, it seems, the protein complex can't form. As a result, cells divide when they shouldn't, resulting in extra growth, and in the case of the mutant mouse embryos, two body axes instead of one. "*Axin* is there to keep the [*Wnt-1*] pathway quiet until the [Wnt] signal comes along," Costantini says.

Inappropriate activation of the Wnt pathway is apparently so dangerous that the cell has evolved multiple ways of keeping it in check. Last year, results from Kimelman and Moon's team suggested that the nuclei of developing frog embryos contain factors that repress the transcription of genes activated by the Wnt pathway. In April, at a cell biology meeting in Heidelberg, Germany, both Mariann Bienz, a developmental biologist at the Medical Re-

search Council Laboratory of Molecular Biology in Cambridge, United Kingdom, and Clevers described two more proteins that interact with Lef1/Tcf proteins much as β-catenin does, but that exert the opposite effect, squelching any gene activation by the transcription factor.

The inhibitory protein identified by the Bienz group is the CREB binding protein (CBP), which is part of the complex of proteins that regulates gene transcription, while the one found by Clevers's team, in collaboration with U.S. researchers, is called groucho because in fruit flies the mutated protein leads to thick bristles reminiscent of Groucho Marx's mustache. "[The] model says that Tcf with [groucho or CBP] is sitting on the target genes and repressing their transcription," he explains. "β-catenin overrides this repression."

Still, failure of the Wnt pathway can be just as catastrophic as too much activity. In the August *Nature Genetics*, Clevers and his colleagues describe how they shut down the pathway in mice by knocking out the gene for Tcf-4. The animals died shortly after birth, apparently because their gut cells failed to maintain a source of immature cells. Instead, the gut lining was made up solely of differentiated cells. "With no Tcf-4 ... you fail to maintain the proliferative [cell type]," Clevers explains. As a result, the digestive tract could not develop properly and thus could not absorb food.

SOURCE: V. KORINEK *ET AL., NATURE GENETICS* **19**, 379 (1998)

β-catenin gone wild

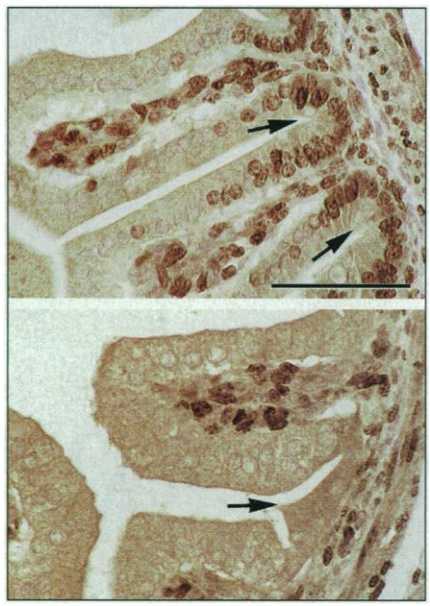
Although these experiments revealed how Wnt's instructions are carried out in the nucleus and also provided information about *APC*'s role as a tumor suppressor, they left a major mystery: the identity of cancer-promoting genes at the end of the Wnt pathway. And that's where the new results from the Kinzler-Vogelstein team come in.

To track down the genes regulated by β-catenin, Kinzler, Vogelstein, and their colleagues at Johns Hopkins used a screening procedure called Serial Analysis of Gene Expression (SAGE), which they developed in 1995 to assess which genes are turned on or off in response to *APC* or other regulators. Because only active genes get transcribed into messenger RNAs, the researchers first made short 15-base DNA copies of all the mRNAs in colon cells lacking a functional *APC* gene. Then, they transferred the *APC* gene into the cells and again made DNA copies, or "tags," of the mRNAs.

By comparing all the tags in the cells with and without the *APC*, the researchers were able to get an idea of which genes were affected by the tumor suppressor's presence. One of the genes whose activity it reduced most was c-*MYC*, which was shut down within 3 hours of the *APC* addition.

"I was surprised," Kinzler recalls. Researchers had found excess c-MYC protein in many types of colon tumors but could never tell whether that excess was a cause or a consequence of the rapid replication of those cancer cells. But now it appears that c-*MYC* activation is a direct consequence of *APC* loss and thus a primary cause of tumor cell replication.

Further evidence for that idea came from experiments in which the Hopkins team attached DNA containing the regulatory region for the c-*MYC* gene to a reporter gene that codes for the luciferase enzyme, which can make cells glow in the presence of another protein, luciferin. When they then introduced the hybrid gene, with luciferin, into human colorectal cancer cells, the cells did in fact glow, indicating that the c-*MYC* regulatory region was picking up an activating signal. But when they added APC protein to these cells, the glow faded—the reporter gene was shut down.



Growth arrest. Normal mouse intestine (top) has pockets (arrows) of growing cells (brown nuclei), but those cells are quiet in mice lacking the Tcf-4 transcription factor (bottom).

As the first clear case in which the direct loss of a tumor suppressor leads to the activation of an oncogene, "[the Hopkins team's result] helps explain the biology of the tumors," comments Harold Moses, a cell biologist at Vanderbilt University in Nashville, Tennessee. Adds SUNY's Marku, "This is an important result for the c-*MYC* people and for the *APC* people."

Important though this finding may be, much of the Wnt-cancer connection remains to be worked out, Kinzler cautions. For one, just as there are multiple controls on the activity of genes at the end of the Wnt pathway, there seems to be more than one way to get to those target genes. Shoukat Dedhar, a molecular biologist at the University of British Columbia in Vancouver, and his colleagues have been studying an enzyme called integrin-linked kinase (ILK) that some reports have implicated as a trigger for cancer. Dedhar's team has found that ILK, which is usually found attached to a cell surface protein called integrin, frees up β-catenin in the cell, but by a different route than does the Wnt signal.

When Dedhar's group added extra *ILK* genes to mouse intestinal epithelial and mammary cells growing in culture, the researchers found that the excess ILK protein produced lowered the amount of E-cadherin. As a result, not only did the cells stop adhering to one another, but the β-catenin that had been bound to the E-cadherin moved into the nucleus, turning on genes that respond to Lef1/Tcf. "It was a pretty thorough demonstration that there may be Wnt-independent ways to activate the Lef1/Tcf pathway," says Kitajewski about Dedhar's results. And ILK may deliver a double whammy, because loss of cell adhesiveness may make a tumor cell more likely to grow into other tissues.

But the surprising convergences that have marked Wnt studies thus far show every sign of continuing. In an upcoming report in the *Proceedings of the National Academy of Sciences*, Dedhar shows that ILK may feed into the WNT pathway after all by inhibiting GSK-3β. "[The pathway] is not as linear as we have thought," says Kitajewski. But he's quite pleased about the progress to date. "We've worked out the skeleton," he says, "and now we're filling in the details."

–ELIZABETH PENNISI

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