Why chemotherapy fails

A single molecule may explain why our most powerful cancer drugs can't always halt tumors

BY RENEE TWOMBLY
Chemotherapy has a lousy reputation. Even the sickest cancer patients are often wary of its many side effects and failure rates.

But new research from Rockefeller University, published last month in the Proceedings of the National Academy of Sciences, may explain why some people don’t respond to the powerful drugs.

The study, led by Archontoula Stoffel, research assistant professor in Hermann Steller’s Laboratory of Apoptosis and Cancer Biology, examined human cells taken from the tumors of a type of non-Hodgkin’s lymphoma. By looking at how specific molecules interact with two different cell growth pathways, Stoffel and her colleagues found a mechanism that may cause chemotherapy drugs to "jam" before they can take effect.

Carbohydrates on trial

A study just launched at The Rockefeller University Hospital will determine whether high fat diets truly work better than high carb diets

BY JOSEPH BONNER
When the Atkins diet first became a national obsession about two years ago, millions of Americans became carb counters. One American, Rockefeller’s Jan L. Breslow, had a different reaction.

After reading a July 2002 New York Times Magazine cover story on the diet, Breslow began digging through medical literature, looking for evidence that the latest diet craze would work any better than any other low-carb diet. “I found that there’s hardly any good, carefully controlled data out there,” says Breslow, Frederick Henry Leonhardt Professor and head of the Laboratory of Biochemical Genetics and Metabolism at Rockefeller.

That won’t be the case for long. Breslow will soon enroll his first volunteer in a rigorous study designed to determine which of two low-calorie diets — one high in fat, the other high in carbohydrates — best reduces abdominal fat in overweight and obese people. Subjects who volunteer for the study will lose, on average, 20 pounds, and scientists will gain a better understanding of the effects of these diets on body composition, risk factors for heart disease and stroke, and overall health.

The Atkins craze has led huge numbers of Americans — Dr. Atkins’ New Diet Revolution has sold over 15 million copies — to abandon low-fat, high-carb diets, which are the basis of the food pyramid recommended by the U.S. Department of Agriculture, the American Heart Association, the American Cancer Society and many other health organizations, in favor of high fat diets. The author of the New York Times Magazine article, science writer Gary Taubes, agreed with cardiologist Robert C. Atkins, the late inventor of the diet, that carbohydrates — the bad guys in the equation — are the real villains. A June 2002 Lancet article concluded that lower carbohydrate diets are better for heart health.

The Atkins craze has also convinced many scientists that it’s time for a new study. "The Atkins craze has led huge numbers of Americans — Dr. Atkins’ New Diet Revolution has sold over 15 million copies — to abandon low-fat, high-carb diets, which are the bases of the food pyramid recommended by the U.S. Department of Agriculture, the American Heart Association, the American Cancer Society and many other health organizations, in favor of high fat diets. The author of the New York Times Magazine article, science writer Gary Taubes, agreed with cardiologist Robert C. Atkins, the late inventor of the diet, that carbohydrates — the bad guys in the equation — are the real villains. A June 2002 Lancet article concluded that lower carbohydrate diets are better for heart health."
Enzyme guidance system. An enzyme called protein phosphatase 1 (PP1), which regulates the flow of signals between nerve cells and helps determine the shapes of their receiving branches, depends on a host of other molecules — around which chromosomal DNA is looped — under a chemical reaction called phosphorylation when their cells divide. Though the purpose of this process is not fully understood, it occurs most dramatically on just two of a cell’s five specific locations in the cell. The researchers have now identified four members of a new class of proteins that bind to PP1 and maneuver it to specific locations in the cell where it is needed. Because the newly discovered protein works by also binding to chromosomal DNA as well as to structural filaments inside the cell called actin, the researchers named them phactr-1 or phosphatase and actin regulators (phactrs) 1 through 4. In studies on flies, Geergerdes’ team detected high levels of phactr-1 in specific sections of the brain: the cortex, hippocampus and striatum — and there was an especially high concentration of the protein in the synapses of nerve cells, which send and receive messages. The study was carried out with colleagues at Yale University School of Medicine. Greengard is the university’s Vincent Astor Professor.

Nature’s backup plan. Scientists have long observed that certain histone-protein complexes — around which chromosomal DNA is looped — undergo a chemical reaction called phosphorylation when their cells divide. Though the purpose of this process is not fully understood, it occurs most dramatically on just two of a cell’s five specific locations in the cell. The researchers have now identified four members of a new class of proteins that bind to PP1 and maneuver it to specific locations in the cell where it is needed. Because the newly discovered protein works by also binding to chromosomal DNA as well as to structural filaments inside the cell called actin, the researchers named them phactr-1 or phosphatase and actin regulators (phactrs) 1 through 4. In studies on flies, Geergerdes’ team detected high levels of phactr-1 in specific sections of the brain: the cortex, hippocampus and striatum — and there was an especially high concentration of the protein in the synapses of nerve cells, which send and receive messages. The study was carried out with colleagues at Yale University School of Medicine. Greengard is the university’s Vincent Astor Professor.

Syndrome X scan. Jeffrey Friedman, Jon Breton and Markus Stoffel have been studying obesity-related diseases on the Micronesian island of Kosrae for the past decade. Now they are using the island population to launch one of the first large-scale genome-wide association studies ever undertaken. Using newly developed “gene chip” technology, the scientists will scan the genomes of more than 2,000 individuals — nearly the entire adult population of the island — in hopes of discovering genetic variations associated with obesity, high blood pressure and diabetes. “We’ve been wanting to do this experiment for a long time, but simply didn’t have tools with the needed power and resolution to get detailed genetic answers,” says Friedman, the university’s Marilyn M. Simpson Professor and head of the Laboratory of Molecular Genetics. Kosrae is an ideal setting for genetic studies because it has a unique mix of Caucasian and Polynesian ancestry and a clear distribution of obesity.

Scents of a gene. The family of genes responsible for producing odor receptors in mice is among the largest of any mammal — about 1,500 genes are devoted to smell. These 1,500 genes encode receptors that are expressed in neurons spread throughout the olfactory epithelium, the lining of the nose that detects odor. Junji Hirotia and Peter Mombaerts have now identified a transcription factor — the third to be discovered in mice — called krox2, which has a positive regulatory role in olfactory sensory neuron development. Hirotia and Mombaerts speculate that krox2 may control both odorant receptor gene choice and olfactory sensory neuron development through distinct mechanisms. Mombaerts is head of the Laboratory of Developmental Biology and Neurogenetics, at the National Academy of Science, June 2004.

Sex and schizophrenia. A team of researchers led by Maria Karayiorgou at Rockefeller and Joseph A. Gogos at Columbia University College of Physicians and Surgeons report on a new susceptibility gene on human chromosome 22. The gene, called ZDHHC8, encodes an enzyme that modifies proteins important for cell-to-cell communication in the brain. Some people with schizophrenia inherit a version of the gene that encodes a defective enzyme. Oddly, female patients with schizophrenia were more likely to inherit the defective gene than males, possibly explaining some of the sex differences observed in the disease. In experiments with mice lacking ZDHHC8, the scientists found that females with one or zero copies of the gene were abnormal with respect to indices of fertility and their ability to process sensory stimuli. The researchers conclude that even modest decreases in the levels of proteins expressed by ZDHHC8 may have substantial effects on behavior and the likelihood of schizophrenia.

Karyayioou is head of the Laboratory of Human Neurogenetics. Nature Genetics, June 2004.

Mutation suppression. In the process of translating genetic instructions into proteins, DNA’s code is fed through two molecular machines — the spliceosome, which reads pre-mRNA to make mRNA, and the ribosome, which assembles proteins from mRNA. Recent research from Mapala Karayiorgou’s Laboratory of Molecular Biology and Biochemistry now suggests that evolution has provided these two machines with similar strategies for coping with imperfect instructions, or mutations in the genetic code. The scientists identified two new forms of a gene, psph, that suppress intron mutations during the two-step process by which the spliceosome deletes specific segments of pre-mRNA. Following several lines of biochemical and genetic analysis, they propose that intron mutations are suppressed by altering the equilibrium between the first and second steps of the process, when the spliceosome changes shape. Mol. Cell, May 7, 2004.

Senseless failure. In the 1960s, David Hubel and Torsten Wiesel showed that if one eye is deprived of visual stimulation, competition arises between the neurons processing signals from the eyes, and the brain’s wiring develops abnormally. Now, Rockefeller’s Peter Mombaerts, working with colleagues at Columbia University, shows that a similar phenomenon occurs in the olfactory system. The scientists used gene-targeted mice to study the maturation of glomerula — structures in the brain’s olfactory bulb where olfactory nerve cells terminate. The researchers found that when the animals are deprived of scents, the glomerula do not fully mature. Furthermore, there is a sensitive period during the mice’s development during which sensory activity influences the organization of the glomerula. The specific timing of this sensitive period varies from one odorant receptor to the next.

Carbohydrates on trial

continued

Although behavioral aspects of dieting are important, Breslow explains, behavioral studies depend on many factors, such as education, physician follow up, availability of certain foods, family situation and economic status, among others. “It is adequately address these issues would require a totally different study design than we have pursued and is really not our expertise,” says Breslow. “The Rockefeller Hospital excels in carefully monitored metabolic studies.” (See “Precision nutrition,” right.)

Breslow is primarily interested in evaluating how much weight can be lost during the course of the study. Visceral fat, which accumulates around the organs in the abdomen, is much more strongly associated with insulin resistance, diabetes, heart disease and stroke, hyperlipidemia and greater total body fat or subcutaneous fat. The study also should help answer some myths regarding high fat diets. For example, it’s commonly accepted, without any scientific evidence, that the low carbohydrate diet have special metabolic effects that promote weight loss. “But the truth is, we really don’t know,” says Breslow: “It’s never been studied.”

Because each study participant can expect to lose about two pounds a week on either diet, for a total loss of 20 pounds by the end of the inpatient period, Breslow calls it a therapeutic study. “The subjects can view their participation as a good start toward maintaining a healthy weight,” says Caline: “They will learn what good portion sizes are and how to eat healthfully. And because the meals are based on natural foods, they will learn the best kinds of food to eat.”

Jan L. Breslow isn’t the first Rockefeller scientist to take on obesity. The Rockefeller University Hospital has been famous since the 1950s for research that requires special diets to study metabolism or nutrients. They’ve gotten quite good at it. “This type of protocol — studying a small number of people with tightly controlled variables — is the hospital’s forte,” says Breslow.

While other research hospitals are weighed down with the beaurocracies associated with routine patient care, Rockefeller is free to focus on research studies initiated by scientists. The bionutrition department, for example, will be critical to Breslow’s research. While other research hospitals are weighed down with the beaurocracies associated with routine patient care, Rockefeller is free to focus on research studies initiated by scientists.

Precision nutrition

Why The Rockefeller University Hospital is uniquely qualified to conduct dietary studies

BY BETSY HANSON

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Working the (immune) system
How four Rockefeller labs are joining forces to cultivate treatments for autoimmune disease

BY LYNN LOVE

As far as Ralph Steinman is concerned, we are alive right now because — among other reasons — our immune system is allowing it.

As you read this newsletter, your T cells, B cells, natural killer cells — each of the immune system's warriors that circulate in your bloodstream — are allowing your body's heart, brain, pancreas, joints and other tissues to exist and function without harassment or interference. This remarkable process is called tolerance.

But in autoimmune disease, that's not the case. The legendary German scientist Paul Ehrlich described the body's capacity to turn on itself as "horror autotoxicus." Ever since Ehrlich's observations at the turn of the 20th century, scientists have tried to figure out what causes the biological system designed to protect your body from disease to revolt against it or break immune tolerance.

Now, for the first time, several Rockefeller University scientists are devising novel and specific ways of treating lupus, type 1 diabetes, multiple sclerosis, rheumatoid arthritis, psoriasis and other autoimmune diseases by unraveling and exploiting the biological mechanisms of tolerance and autoimmunity.

"The progress is not only an example of how science, creatively practiced, can shed light on the body's most complex and mysterious workings — it's also a model of how collaboration among Rockefeller labs can solve problems that might be too big for a single lab to tackle. Four of the university's seven immunology labs, headed by Steinman, Jeffrey Ravetch, Michel Nussenzweig and Alexander Tarakhovsky, all part of the university's Christopher H. Brown Center for Immunology and Immune Diseases, are tackling different but related pieces of the autoimmunity puzzle. The interactions are a testament to how Rockefeller's small size and flat administrative structure can make the best use of limited resources to answer big questions. The labs have formed a de facto working group what's revealed in one lab is often incubated in the next."

A key element of the understanding they are developing is one of active maintenance of tolerance on a daily basis, in the "steady state." As long as the immune system's killer cells tolerate the body's tissues, health is preserved.

"This tolerance is literally what keeps us alive," says Steinman, head of the Laboratory of Cellular Physiology and Immunology. "Studies in immunology have most often emphasized the immune system's role in defense against infections and tumors, but tolerance is just as critical, even in the steady state. If one could learn how to induce authentic immune silencing, or tolerance, to the causes of autoimmune disease and allergy, it would constitute a revolution."

The spiny dendritic cell, discovered by Steinman and

Eluding lupus

In the case of lupus, the immune system attacks not a single organ but the skin, joints, blood and kidneys. As many as 1.5 million Americans have been diagnosed with lupus.

The problem is antibodies. Antibodies, Y-shaped proteins that lead the immune system to specific invaders, start forming in humans from infancy and hang out inside the body. When appropriate, they bind to microbes and other foreign material, called antigens. This connection between an existing antibody and an antigen starts a process called clonal expansion, in which the body's B cells produce great numbers of antibodies. Some of these new antibodies will have a tighter fit with the existing antigens than the original antibody did.

With bacteria or other invaders, a tight fit with an antibody makes the pathogen easier to identify and destroy. But during clonal expansion, antibodies are also created that bind to the body's own cells (see illustration, right). These antibodies are known as autoantibodies. Ideally, the body's immune system tolerates these autoantibodies. But when it doesn't, the results are disastrous.

"Autoactivity is not the exception, it's the rule," Ravetch says. "Yet the body maintains tolerance and autoimmunity is held off why?"

One of the immune system's antibodies, known as immunoglobulin G or IgG, actually controls the activities of other antibodies that are prone to autoactivity. In most cases, IgG inhibits the potential onset of the autoimmune cascade we know as lupus and it performs this role via the Fc receptor that forms in, and every other antibody's, Y shape.

But not always. In 2000, Ravetch, who is an expert on the Fc receptor, and postdoc Sibra Bolland discovered that a defect in the IgG Fc receptor will cause spontaneous autoimmunity in mice that are genetically predisposed to lupus.

"Many people who could develop lupus never do," says Ravetch. "But the animals we studied broke tolerance and developed full-blown lupus. So we immediately wanted to know how this inhibitory receptor contributes to the maintenance of tolerance."

Once Ravetch and his colleagues further characterized IgG's inhibiting role in lupus, they may be able to develop a means of repairing faulty Fc receptors that lead to the onset of the disease.

Tolerance in the face of disease

In the case of autoimmune diseases like type 1 diabetes, psoriasis and other autoimmune diseases by unraveling and exploiting the biological mechanisms of tolerance and autoimmunity. The progress is not only an example of how science, creatively practiced, can shed light on the body's most complex and mysterious workings — it's also a model of how collaboration among Rockefeller labs can solve problems that might be too big for a single lab to tackle. Four of the university's seven immunology labs, headed by Steinman, Jeffrey Ravetch, Michel Nussenzweig and Alexander Tarakhovsky, all part of the university's Christopher H. Brown Center for Immunology and Immune Diseases, are tackling different but related pieces of the autoimmunity puzzle. The interactions are a testament to how Rockefeller's small size and flat administrative structure can make the best use of limited resources to answer big questions. The labs have formed a de facto working group what's revealed in one lab is often incubated in the next."

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The spiny dendritic cell, discovered by Steinman and
collaborators in 1973, in a nomen for clues about how the immune system functions: the specialized cell carries out both the protective work of the immune system and its preventive work of maintaining tolerance. This is because the dendritic cell is what's known as an antigen-presenting cell. It picks up foreign molecules — bits of the invading bacteria, for example — then evaluates them and relays messages about how best to respond to other immune system cells such as T and B cells that then carry out the alert work. Through this biologically diadic process, dendritic cells can recognize an enormous variety of antigens and either encourage or subdue an immune system attack.

The scientific community was not prepared to consider a new cell type in the immune system when Steimman made his famous discovery 30 years ago. "We discovered the dendritic cell because we had new microscopes and other cell biological methods to pursue things we saw that did not fit into accepted categories," says Steimman, who is the university's Henry G. Kunkel Professor. "When we discovered it, we could not envisage how important the dendritic cell would be in understanding both the immunity and tolerance aspects of the immune system." It wasn't until the 1990s that the dual role of the dendritic cell was fully appreciated. Soon after the dendritic cell discovery, Michel Nussenzweig, now Rockefeller's Sherman Fairchild Professor and head of the Laboratory of Molecular Immunology, came as a graduate student to the laboratory then headed by Steimman and the late Zareli Colin. "It was a perfect time for me, a curious young medical student, to enter the field. The dendritic cell seemed like the most exciting and natural project to devote myself to," says Nussenzweig.

Nussenzweig has since emerged as a leader in his own right in the field of autoimmunity. In 2001, for example, he and graduate student Daniel Hawiger proved that the dendritic cell is as responsible for establishing tolerance to the body as it is to getting the body to eradicate pathogens — a finding that cleared the way for dendritic cells to be exploited in the treatment of autoimmune disease.

However, the dendritic cell alone does not tell the whole story. In fact, it's just one of several checkpoints the immune system has developed to ensure that its force isn't unleashed on the wrong target.

Think of it as an electric circuit: in order for a circuit to be completed, a series of switches need to be closed. "At a basic level, the immune system is a checkpoint system," says Jeffrey L. Ravetch, Theresa and Eugene Lang Professor and head of Rockefeller's Laboratory of Molecular Genetics and Immunology. "From the earliest days of B cells to the way that T cells are informed by the dendritic cell, the body as it is to getting the body to eradicate pathogens — a finding that cleared the way for dendritic cells to be exploited in the treatment of autoimmune disease.

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But remarkable new research from Steimman's lab now shows that it may be an imbalance of immune system T regulatory cells that triggers the onslaught. These cells work as suppressors: they turn off the body's immune response. Steimman's study, which was published in the June 7 issue of the Journal of Experimental Medicine, shows that dendritic cells can be used to expand functional T regulatory cells, which can actually reverse the course of type 1 diabetes in mice.

"Instead of silencing the attackers directly, we learned how to generate another type of cell, a suppressor cell, which essentially turns off the attackers," says Kristin Tarbell, a postdoctoral associate in Steimman's lab. "At that point, it's basically a numbers game. At the onset of the disease there are not enough of the regulatory cells to suppress the immune response against the body's insulin-producing pancreatic islet cells. By putting the right number (in the case of mice, 5,000 to 50,000 regulatory T cells) in the right place, the researchers arrested the process (see illustration, right).

Steimman and Tarbell's study used mice that were genetically predisposed so that their suppressor T cells, once activated, would home in directly on the pancreatic islet cells. Now, the scientists need to run the same experiment in mice with normal T cells. This critical next step will determine whether the research can be moved to clinical studies in humans.

Even now, the findings prove an important biological principle that could lead to prevention of type 1 diabetes in humans: autoimmunity can be reversed if the immune system's mechanisms for tolerance — recognition and acceptance of the body's own cells — can be repaired, and dendritic cells mediate this repair.

Take it a step further, and the research could have implications beyond prevention of the disease. Islet cell transplantation, for example, is still experimental technology, is far from foolproof. "At the moment, the problem with islet cell transplants is that the process that destroyed the first set will destroy the second," Tarbell explains. But with the ability to restore balance in the immune system, islet cell transplant could succeed.

An additional checkpoint was discovered by Nussenzweig and postdoc Heidi Wardeman in 2003. Their research found that a majority of early immune system B cells — which are important to antibody production during infection — are self-reactive. If allowed to mature, these misguided B cells would predispose the body to severe autoimmune disease, most likely lupus. Yet because of an immune system checkpoint, these self-reactive B cells are killed off before they fully mature in the bone marrow.

Another series of checkpoints, identified by Steimman's Alexander Tarakhovsky, head of the Laboratory of Lymphocyte Signaling, are signaling proteins, such as one discovered by Tarakhovsky and postdoc Ingrid M. Mckenzie called protein kinase C-delta, which can establish or foil the steady state of tolerance.

"We can almost think of the immune system as a symphony, or a cascade, of signaling, which brings about certain changes to individual cells and their ability to recognize antigens," says Tarakhovsky. "When that signaling fails to maintain tolerance, the symphony takes on a dissonance that it normally avoids."

Understanding the checkpoints — and being able to hear the symphony — will eventually lead to new ways of controlling the immune system when it misfires and may eventually help combat some of our most perplexing diseases.

Halting diabetes

When an out-of-balance immune system attacks the pancreas, the result is type 1 diabetes. Over 5 million people worldwide rely on insulin injections to metabolize the sugars that their own bodies can no longer process.

But remarkable new research from Steimman's lab now shows that it may be an imbalance of immune system T regulatory cells that triggers the onslaught. These cells work as suppressors: they turn off the body's immune response. Steimman's study, which was published in the June 7 issue of the Journal of Experimental Medicine, shows that dendritic cells can be used to expand functional T regulatory cells, which can actually reverse the course of type 1 diabetes in mice.

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Deaf fish yields gene linked to blindness

Zebrfish that can't hear provide clues to deafness and a model for studying a human eye disorder

BY TIEN SHUN LEE

For most men, hair is something you want more of on your head and less of in your ears. So you're not necessarily so glad for Rockefeller's Jim Stoffel.

Hudspeth studies the tiny bundles of hair, burled deep inside our ears, that translate sound waves generated by mechanical forces — the drawing of a bow across strings, the crashing of a car against a wall — into electrical signals that can be processed by the brain. Now research in his lab has identified a gene critical to the process by which these hairs develop.

Stoffel's Laboratory of Sensory Neurosciences has long studied bul- lfish, whose extra-large ear hairs are well suited for analysis. In recent years, however, Hudspeth's team has also been focusing on zebrfish, which are excellent for genetic studies. The scien- tists wanted to find a deaf fish that would lead them to key genes and molecules involved in hearing.

Six years ago, Hudspeth, who is the F. M. Kirby Professor and director of the F. M. Kirby Center for Sensory Neurosciences, and Catherine Starr, then a graduate student in Hudspeth's lab, began the painstaking process of screening thousands of genetically mutant zebrfish. To find out whether or not the fish could hear, Starr repeatedly tapped on the sides of their tanks. Normal zebrfish would turn away from the sound. Stoffel was looking for a fish that did not respond.

When she eventually found one, Starr paired the deaf fish with a normal fish, and then used genetic mapping techniques to identify a mutant gene in deaf offspring. Because the mutant gene is similar to a human gene called Chordoma, Stoffel named her fish chordoma, or chm.

To confirm she'd found the right gene, Starr inject- ed the RNA product of the chm gene into fertilized fish eggs. She found that 50 percent of genetically mutant fish, which would normally have ended up deaf, were "rescued" by the chm RNA. Like normal fish, they quickly swam away when their container was tapped.

The cancer the researchers used as their model is known as mucosa-associated lymphoid tissue lymphoma (MALT lymphoma), which consists of tumors that origi- nate from cancerous growth of immune cells. MALT lymphoma most often occurs in the stomach and usually arises when immune system B cells respond to infection by bacteria. This infectious immune response, tells cells to grow, and abnormal activation of the NF-κB immune responses, tells cells to grow, and abnormal activation of the NF-κB pathway, which normally helps clear cells that are damaged, then produces systemic inflammatory responses, "That makes sense because lymphoma is a tumor of the immune system and abnormal activation of the NF-κB pathway, which is normal- nately involved in setting up an immune response, leads to abnormal proliferation," Stoffel says.

More startling was the scientists' finding that five genes were known to be involved in a cell suicide process called apoptosis. When they checked with databases of gene products, the researchers found that three of these five have been shown to block the function of the five p53 protein.

“Than surprised us,” says Stoffel. “We began to think that maybe the fusion proteins work in a similar way, by turning on NF-κB and inhibiting p53. There have been suggestions in previous research that these pathways might be interrelated, but no one has seen that in a model of cancer.”

To check whether fusion proteins inhibited p53, Stoffel exposed cells to ultraviolet radiation, which is known to stimulate the activity of p53 and kill cells. “But our cells did not die. That meant something was controlling the activity of p53,” Stoffel says.

Next, the researchers treated the cells with molecules on which they knew would block NF-κB and again exposed them to ultraviolet radiation. This time, the cells died.

“The NF-κB pathway, which is the hallmark of all immune responses, tells cells to grow, and abnormal activa- tion of this pathway leads to abnormal proliferation and cancer,” Stoffel says. “If this pathway also inhibits p53, which normally helps clear cells that are damaged, then you can't eliminate these cells.”

The team concluded that p53 was not inhibited by the NF-κB pathway. The fusion proteins were promoting cancer development by encouraging cell growth through the NF-κB pathway, which, in turn, repressed p53 and inhibited cell death.

The results suggest different ways to inhibit the NF-κB pathway for cancer control. “Many people are now thinking of making NF-κB, but that produces systemic effects in patients because this pathway plays such an important function in normal cells. It is active in disease but also needs to be intact for normal immune and inflammatory responses,” Stoffel says.

The goal now is to identify molecules downstream of the NF-κB pathway that could be targets for new drugs able to activate tumor suppressor genes without upsetting the body's other cells.
It’s the biochemist’s twist on the old light bulb joke: how many proteins does it take to activate a gene? In the case of p53, a widely-studied gene linked to tumor suppression, the number keeps growing.

Rockefeller scientists now know that as many as five are involved in just the first step of the activation process. “The regulation of gene expression is one of the most important and actively studied areas in biology today, and an understanding of the central role of histone modifications in gene activation has enormous implications for understanding both normal and abnormal cellular processes,” says Roeder, head of the Laboratory of Biochemistry and Molecular Biology and last year’s recipient of the Albert Lasker Award for Basic Medical Research. “Tails of transcription, gene’s DNA.”

“It’s the biochemist’s twist on the old light bulb joke: how many proteins does it take to activate a gene?”

As many as five proteins are required to activate a single gene, “a target gene wrapped up in a chromatin structure with histones.”

“There have been many studies correlating histone modifications and transcriptional regulation, but none of the studies directly showed that histone modification itself actually regulates transcription,” says An. “Our results show — as was generally assumed — that chemical modifications of histones indeed are required for the activation of a gene transcription.”

To show that this is the case, scientists treated cells with UV light, which induces DNA damage and causes GADD45 to be highly expressed, and watched the recruitment of cofactors to the site of DNA damage. The results showed that the proteins followed a specific order: first, p300, then PRMT1, CARM1 and PRTM1, which co-activators are required to turn genes on.

“Structure of a DNA attack

After two years and hundreds of gallons of buffering solution, scientists at C. Eric Stebbins’ laboratory have unraveled the atomic structure of a protein that disease-causing bacteria use to attack human DNA.

“The image they obtained of the devastating toxin CDT (right) will help scientists understand the activity of this potential carcinogen and to design new drugs to fight cancer and other diseases that are linked when the toxin enters a cell and breaks that cell to stop dividing and die.”

“Many CDTox-containing bacteria are discovered each year,” says Stebbins, head of the Laboratory of Structural Microbiology. “Many of these bacteria cause very different kinds of disease and colonize different tissues, but they all have CDT To me, that argues that it’s playing an important role.”

“Structure of a DNA attack

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The opsinless mind. Olfactory neurons — visualized here in blue — normally express an odorant receptor molecule called MT1 and have extensions that continue into a single spot, a glomerulus, on the surface of the brain's olfactory bulb (above). Rockefeller's Peter Mombaerts generally forced the neurons to instead express a different receptor, the β2 adrenergic receptor, and found the projections form a glomerulus, though in a different spot (left).

Despite the name, most of the bulb's millions of neurons, olfaction appears to be one of the simplest brain systems, the Rockefeller researcher says Odor molecules entering the nasal cavity travel to the olfactory epithelium, a patch of tissue almost level with the eyes. There, thousand of receptors on the hair-like cilia of nerve cells embolded in the tissue. Neurons that serve those receptors each send an axon back to one of two olfactory bulbs in the brain, and “like-minded” axons — those originating from neurons that link to the same odorant receptor — terminate together into brain structures known as glomeruli.

When an odor molecule interacts with a receptor, it impels the neuron to the glomeruli, some 2,000 of which reside within each olfactory bulb. The glomeruli act as relay stations, processing nerve signals from the olfactory epithelium to other regions of the brain. About 5,000 axons form a glomerulus and branch into 50,000 axon terminals that synapse with the dendrites of 25 or so second-order neurons, all in the space of a parachute, with a diameter of one-tenth of a millimeter. The question for Feinstein and Mombaerts: How do all these axons find their way to such a tiny and precise spot in the brain?

The prevailing view is that axons navigate to glomeruli via positional cues or signaling information secreted by cells in the olfactory bulb, says Feinstein. People view glomeruli as existing structures, target which axons were aiming for. But glomeruli are not seen when the olfactory system begins to develop. How can a developing axon be drawn to a structure which does not yet exist? In 1996, Mombaerts published findings that odorant receptors were capable of organizing “like” axons into glomeruli. It was a major hunt. But we had no model for how they could do that,” Mombaerts says. “It was a bizarre notion that receptors could somehow be involved in telling axons where to navigate. As far as anyone knew, the only role odorant receptors had was to smell. We had no proof of another function.”

The Cell studies now detail that proof. The first paper leaves off to ask how odorant receptor expression modulates axonal identity. The team genetically substituted the odorant receptor with an unrelated receptor, a G-protein coupled receptor (GPCR) called the β2 adrenergic receptor. “The expressed β2 adrenergic receptor also supported the formation of glomeruli, which indicates that this receptor, which has nothing to do with the smell system, can also work to guide an axon,” Mombaerts says. “That further suggests that the olfactory bulb isn’t just attracting axons.”

The conclusion: “Our experiments reveal that the odorant receptors exist not just at the ends of nerve cells in the olfactory epithelium, but also along their entire length.” Mombaerts says. “We hypothesize that axons with odorant receptors appear to interact stronger with axons carrying similar odorant receptor structures and repel those that are different. As a result, the system self-organizes such that axons eventually coalesce into like-minded bundles.”

The second Cell paper, by Feinstein, Mombaerts and research associates Thomas Bozza, Ivan Rodriguez and Anna Vassalli, picks up where the first paper leaves off to ask how odorant receptor proteins modulate axonal identity. The team genetically substituted the odorant receptor with an unrelated receptor, a G-protein coupled receptor (GPCR). The β2 adrenergic receptor has been used to change axonal identity: “This paper confirms several predictions of axonal identity,” Feinstein. “We show that odorant receptors, including the β2 adrenergic receptor we used for the gene swap, are expressed in extending axons. We further show that these GPCRs may also promote axon outgrowth, and speculate that these processes of axon identity and axon outgrowth may be a more general phenomenon throughout the brain governed by what similar protein.”

From an evolutionary perspective, the new model makes perfect sense. “The olfactory system has to be very plastic to accommodate new genes encoding odorant receptors and new populations of neurons,” Mombaerts says. “If you have a rigid view that axons are attracted by existing cues, it would be much more difficult to explain how these new populations of neurons wire themselves.”