Winter 1995

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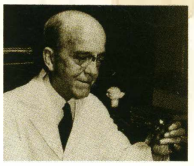
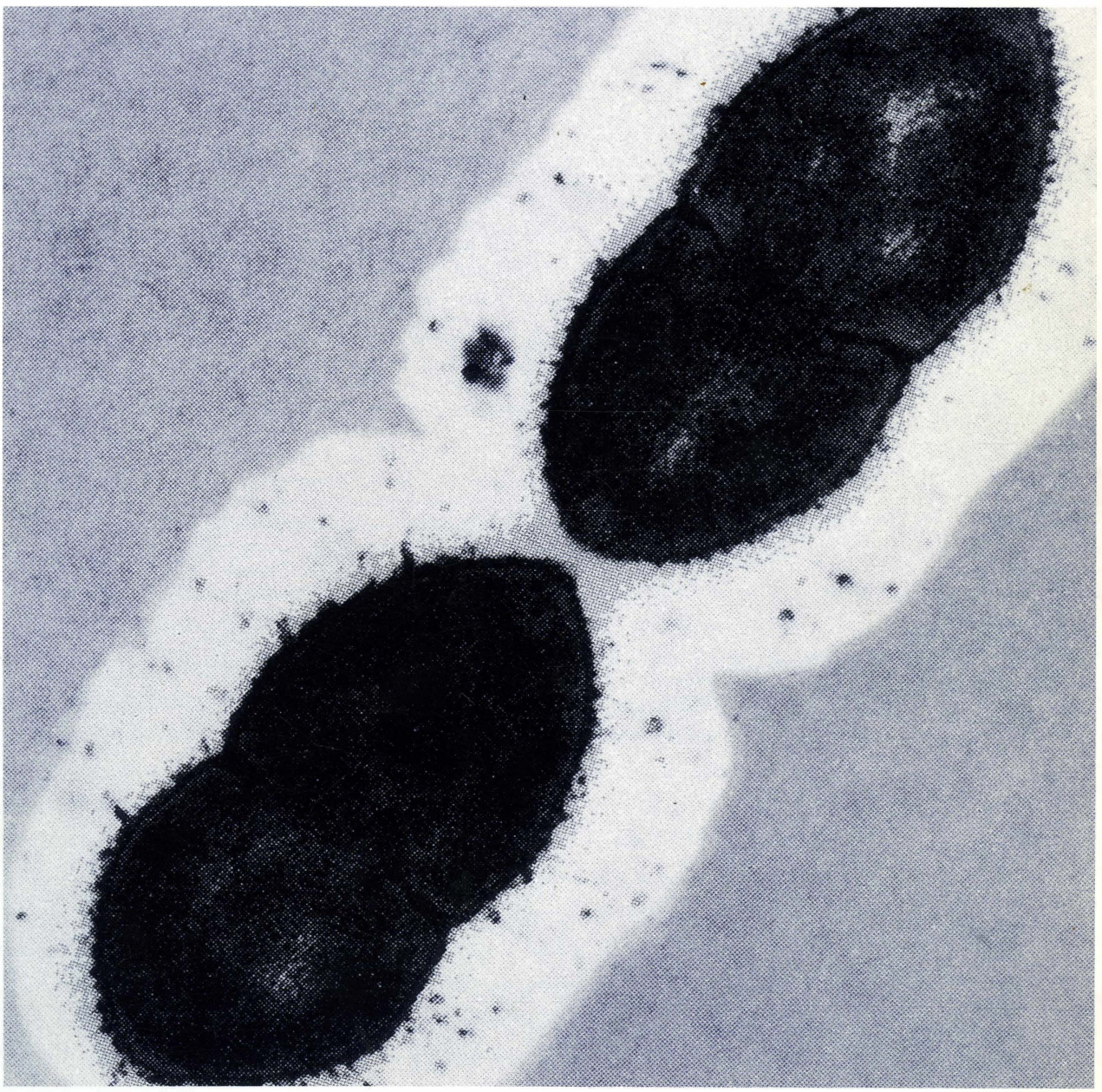


THE MODERN LEGACY OF THE AVERY LAB



TRANSFORMATION

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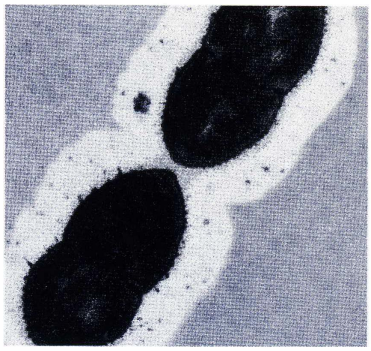

TRANSFORMATION:
The Modern Legacy of the Avery Lab

This cover of *Search* shows the pneumonia bacteria, surrounded by a halolike protective capsule (magnification: 25,000). Beginning in 1913, Oswald Avery, a leading pneumonia researcher, showed that different protective capsules—each a polysaccharide with a distinct chemical identity—surround different strains of pneumococcus, and are essential for virulence. Take the capsule away, and the bacterium is rendered harmless. When, in 1928, researcher Fred Griffith showed that one type of bacteria (naked and harmless) can be transformed into another (encapsulated and lethal), Avery set about discovering the identity of this mysterious chemical substance which caused the transformation. Sixteen years later, joined by Colin MacLeod and Maclyn McCarty, the Avery team published its landmark 1944 paper showing that DNA was the transforming principle—a substance that could cause a heritable change of bacterial cells.

The Avery lab's work did not just uncover the genetic secret behind transformation—it was a seminal "transforming" moment in biology. For it was this revolutionary finding that set the stage for Francis Crick and James Watson's dramatic unraveling of the double-helical structure of DNA in 1953 and started a modern revolution—in genetics, molecular biology, infectious disease—that continues to transform our lives, a transformation to which this issue of *Search* is dedicated.

The Editor

Winter 1995

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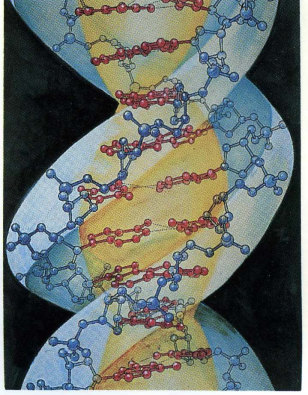
In 1994, The Rockefeller University celebrated the 50th anniversary of what the great immunologist Peter Medawar called "the most interesting and portentous biological experiment of the 20th century": the discovery that DNA carries genetic information. This revolutionary finding by Oswald Avery, Colin MacLeod and Maclyn McCarty, the fruit of fifteen years of painstaking work in Avery's pneumonia-research laboratory at The Rockefeller Hospital, was first published in *The Journal of Experimental Medicine* on February 1, 1944. As the late Lewis Thomas wrote: "This single discovery opened the way into the biological revolution which continues to transform our view of nature in the most intimate details, and continues as well to cast up, in its wake, one biotechnology after another for the comprehension and, it can be hoped, the reversal of human disease processes."

In this issue of *Search*, we trace both the historical roots of the Avery laboratory's 1944 paper, and an array of remarkable new discoveries that have flowed from it. "The Legacy of Avery" shows how Avery's pneumonia research program led to the DNA discovery, and how our newfound ability to read and manipulate DNA is being used by Dr. Vincent Fischetti in the continuing fight against infectious disease microbes. "The Bugs Are Back" describes Dr. Alex Tomasz's research and public health efforts in understanding and designing new therapies against antibiotic-resistant bacteria, one of the major medical problems in the world today. "Following the Thread of Life" profiles the work of five leading young DNA researchers at the university—Drs. Stephen Burley, Frederick Cross, Stephen DiNardo, Jeffrey Friedman and Titia de Lange. Dr. Friedman's recent and much-heralded discovery of the first gene linked to the regulation of body weight, providing a new foundation for the study of the molecular basis of human obesity, is a shining example of how modern DNA science is changing the face of medical research. "Opening Pandora's Box" discusses some of the ethical issues arising from our newfound knowledge in the context of Dr. Nancy Wexler's quest for the Huntington's disease gene. Highlights from our year-long celebration of Avery, MacLeod and McCarty's revolutionary finding are pictured in a special photo essay.

A particularly exciting highlight occurred on September 30, when Professor Emeritus Maclyn McCarty received the Albert Lasker Special Public Health Award—only the fifth time in the distinguished history of the Lasker Awards that this special recognition has been given. Like Avery, Mac is a famously modest man, who would readily echo a favorite saying of his mentor's: "Apply the brakes when tempted to blow your own horn." One of the great pleasures of this anniversary year has been the opportunity for the university community and scientific world to blow the trumpet for Mac, and for the late Oswald Avery and Colin MacLeod.

Torsten Wiesel
President



From Infectious

Rockefeller University Archives

Above, Members of the Avery laboratory, about 1932. (*From left to right*), Seated: Thomas Francis, Jr., Oswald Avery, Walther F. Goebel. Standing: Edward E. Terrell, Kenneth Goodner, René J. Dubos, Frank H. Babers.

It was called the "crisis."

Ten or twelve days after the first signs of sickness, the pneumonia patient's fever would rise precipitously; the bacterial infection of the lungs would peak; the patient's immune system would either launch a successful attack against the invading bacteria, or the patient would die. One-fifth of pneumonia victims did not survive the crisis; four-fifths recovered. At the turn of the 20th century, when The Rockefeller Institute for Medical Research was established, lobar pneumonia was the nation's leading cause of fatality. The great English physician William Osler called pneumonia "Captain of the Men of Death." Pneumonia was, Osler said, "a self-limited disease which can neither be aborted nor cut short by any means at our command."

For Oswald T. Avery, the key to the pneumonia crisis was contained in small vials of sugar. Between 1913, when he was recruited to work on pneumonia therapy at The Rockefeller Hospital, and the late 1920s, Avery and several brilliant collaborators discovered that these complex sugars, or polysaccharides, composed the protective capsules surrounding pneumococci bacteria, shielding them and preventing their engulfment by bacteria-eating immune cells called phagocytes.

Different types of pneumococci were protected by capsules made of different polysaccharides. Avery's initial research at Rockefeller was aimed at isolating animal antibodies against the capsules of different pneumococcal types, which could then be injected into human pneumonia patients before they reached the crisis. Until the advent of antibiotics, this "serum therapy" was medicine's only weapon against lobar pneumonia.

This work helped usher in the era of antibiotics,

The Legacy of Avery:
Disease to DNA

by Geoffrey Montgomery

and in the process established the paradigm for the modern study, diagnosis and treatment of all infectious diseases. And it was a mysterious phenomenon involving this sugary capsule that also led Avery, Colin MacLeod and Maclyn McCarty to what the Nobel Prize-winning immunologist Peter Medawar has called "the most interesting and portentous biological experiment of the 20th century"—the demonstration that genetic information is carried by DNA.

Launching the Antibiotic Era

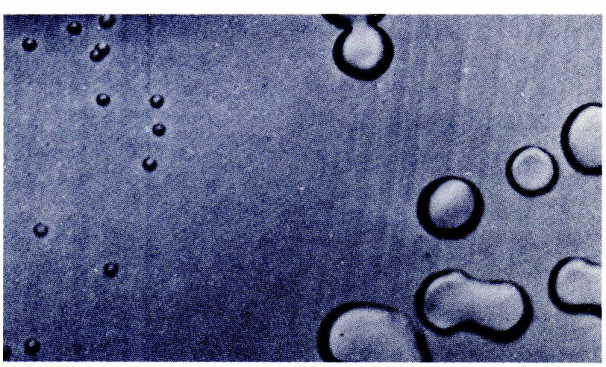
In his account of this discovery, *The Transforming Principle*, Maclyn McCarty has described Avery's tenacious genius: "an uncanny ability to ask the right questions [about a scientific problem] and a dogged persistence in finding the answers." Rockefeller's René Dubos also described this talent in his account of his first meeting with Avery in 1927, when the two discovered their intersection of interests over lunch. Dubos told Avery how he had discovered enzymes secreted by soil bacteria that decompose cellulose, the polysaccharide that gives plants their stiffness. Avery immediately remarked that this plant polysaccharide was related in chemical structure to the polysaccharide composing the capsule of the most deadly of pneumococcal strains, called Type III.

"As if by a casual gesture, but in fact deliberately," wrote Dubos, "Avery took from the right-hand drawer of his desk a little tube containing a white powder, labeled in his neat handwriting SSSIII, and shook it in front of me." Still shaking the capsule, Avery said to Dubos: "This is the polysaccharide of which the capsule is made. It is completely resistant to the body enzymes and to all other enzymes we have used. ...If only we knew of a way to decompose it with an agent mild enough to be used in the body—an enzyme, for example—much could be learned about pneumococcal infections."

Dubos found himself fascinated by this challenge. A year later, in the summer of 1928, he discovered an enzyme made by soil bacteria in the cranberry bogs of New Jersey that could specifically break down the Type III pneumococcal capsule. The enzyme, called SIII, cured pneumonia in experimental mice, but it could not be used in humans because it had to be injected directly into the lungs. The discovery did, however, lead to the isolation of more easily administered antibiotics, and it launched the antibiotic era.

DNA: The Transforming Substance

Also in 1928, the British medical scientist Fred Griffith discovered a mysterious transformation that would lead to the most spectacular of the Avery laboratory's achievements. Griffith injected into mice Type II pneumococci that had lost the ability to make a protective capsule; these naked bacteria were harmless, easily swallowed by immune cells. Griffith simultaneously injected killed Type III pneumonia bacteria into the mice—also harmless. Yet the mice died; and from their bodies Griffith recovered living, and viru-



Above, Transformation of pneumococci from harmless, denuded form (*left*) to virulent, encapsulated form (*right*) studied by Avery, MacLeod and McCarty and published in the 1944 landmark paper.

lent, Type III bacteria.

The dead Type III pneumococcus had not been miraculously resurrected. Rather, as the Avery lab soon established, the dead Type III bacteria were transferring some unknown chemical, dubbed "the transforming substance," to naked Type II cells that allowed them to grow a protective Type III capsule. And the transformed cells passed on this new property to their descendants.

From a purified transforming extract, Avery wrote, "there separates out a fibrous substance which on stirring the mixture wraps itself about the glass rod like thread on a spool."

As Avery had earlier pursued the chemical composition of the capsule responsible for pneumococcal virulence, so he and two young colleagues, Colin MacLeod and Maclyn McCarty, now sought between 1934 and 1944 the chemical identity of the substance responsible for this transformation.

"Some job—full of heartaches and heartbreaks," wrote Avery in 1943 to his brother Roy, a bacteriologist at Vanderbilt. "But at last perhaps we have it." From a purified transforming extract, Avery wrote, "there separates out a fibrous substance which on stirring the mixture wraps itself about the glass rod like thread on a spool." The threadlike transforming substance was deoxyribonucleic acid, or DNA—the thread of life.

From DNA Back to Infectious Disease: The Modern Synthesis

Avery, MacLeod and McCarty published their revolutionary finding in the February 1, 1944 issue of *The Journal of Experimental Medicine*. Fifty years later, this discovery has transformed biological science. Yet humankind has remained plagued by infectious diseases, from tuberculosis to the AIDS virus to a recent outbreak of deadly streptococcal strains. And it is only relatively recently that the two strands of the Avery lab's research—in infectious disease and DNA—are being wound together like the two strands of the double helix itself.

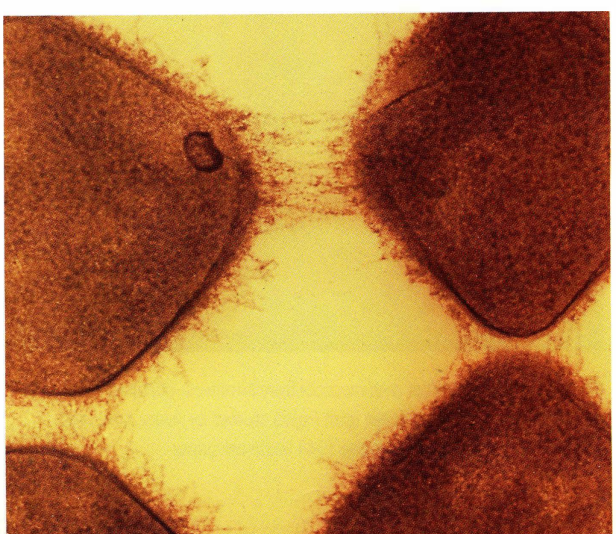
Studies by Rockefeller scientists and others have made it clear that the kind of DNA-mediated transformation studied by Avery and his colleagues was no laboratory curiosity; it is one of several natural mechanisms bacteria use in their constant struggle for survival. As antibiotics course through the bodies of antibiotic-treated humans and animals, the bacteria living inside evolve and resist these drugs. [See page 20 of this issue.]

Antibiotic resistance has highlighted the need for vaccines against bacterial pathogens. And here also the work of Avery and his colleagues continues to serve as a guiding light. In the 1940s, Michael Heidelberg and MacLeod—two descendants of the Avery lab then at Columbia University and New York University, respectively—developed the first pneumonia vaccine. The vaccine was made up of polysaccharides isolated from eight types of pneumococci. Yet with the advent of antibiotics, the use of this pioneering vaccine was abandoned, although Robert Austrian of the University of Pennsylvania School of Medicine, who had worked with MacLeod at NYU, later led a heroic scientific and public health effort to develop an improved version. And, in the late 1960s, Rockefeller Professor Emil Gotschlich also pioneered a new type of polysaccharide vaccine—against the bacteria that cause meningitis.

Polysaccharide vaccines work by inducing the body to generate antibodies that bind to specific types of bacterial surfaces. Such vaccines do not evoke so-called "cellular immunity," however, which is mediated by specialized immune cells called T lymphocytes. While adults can overcome bacterial infection solely with antibodies (produced by B cells of the immune system), children require a T cell response as well. T cells are unable to recognize a polysaccharide in isolation; the polysaccharide must be linked, or "conjugated," to a larger molecule, such as a protein. The first experimental conjugate vaccine, which chemically joined a crucial piece of pneumococcus polysaccharide with the albumin protein of egg white, was pioneered by Walther Goebel and Avery in 1931.

Yet it was not until 1987 that the first conjugate vaccine, developed by John B. Robbins and his colleagues at the National Institutes of Health, was licensed for clinical use. The vaccine was targeted against Hemophilus influenza Type B, which infects 1 of every 250 children in the United States, killing 10% of those infected, and leaving another 30% with permanent afflictions such as blindness or deafness.

Vincent Fischetti



Left, M proteins, which shield Group A streptococci from immune cells, appear as hairlike filaments covering the surface of bacterial cells.

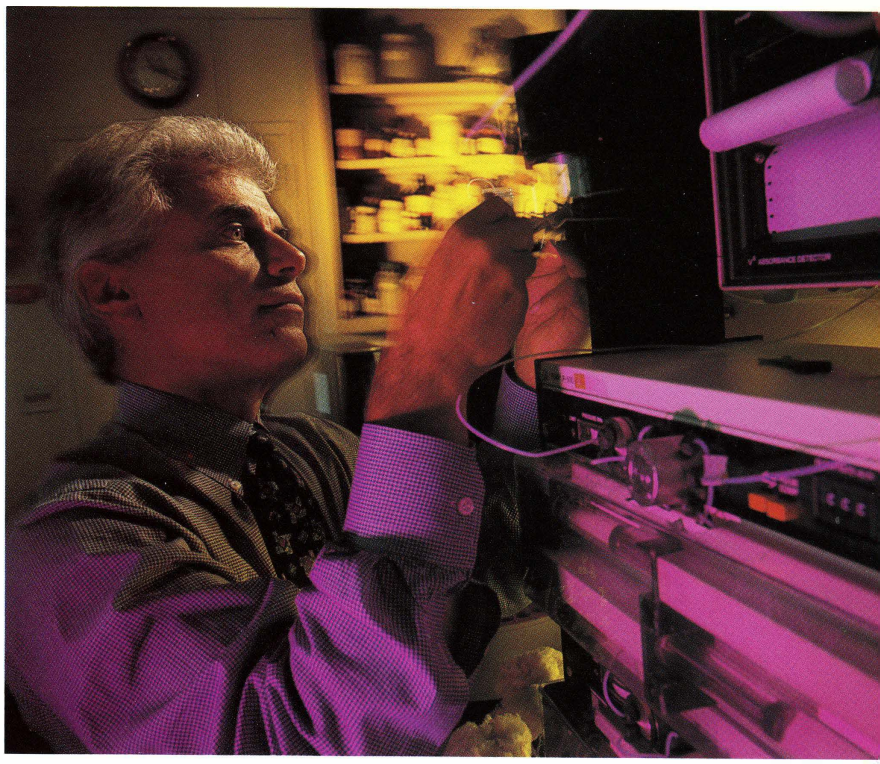
The basis of the vaccine, says Robbins, was laid in the paper published in 1931 in *The Journal of Experimental Medicine* by Goebel and Avery.

No example better illustrates how the twin strands of the Avery lab's research in infectious disease and DNA have been fused in modern research than the work of Rockefeller Professor Vincent Fischetti on streptococcal vaccines. Streptococci cause not only strep throat, but rheumatic fever—and in rare cases of a toxin-producing strep bacteria infected by a toxin-producing virus, sudden necrosis of the body's tissue and even death. Following the pioneering trail of Avery's colleague at Rockefeller, Rebecca Lancefield, Fischetti and his co-workers have studied how streptococci protect themselves from human immune cells through surface molecules called M proteins. M proteins play an analogous role to the polysaccharide capsule shielding pneumococci; they protect the bacteria from engulfment. But the 80 known streptococcal strains possess 80 different types of M proteins.

After cloning the DNA encoding the M protein, Fischetti and his colleagues discovered both a region of the M protein that was shared among the 80 different streptococcal M proteins and a region common among nearly all bacteria classified as gram-positive, which includes the pneumococcus and the bacteria responsible for staphylococci infections. This latter conserved region serves to anchor proteins like the M protein on the surface of the gram-positive bacteria. By genetically splicing this anchor sequence along with the M protein common region into a harmless bacteria that normally colonizes human teeth and gums, Fischetti and his colleagues have been able to design a recombinant DNA vaccine that elicits antibody production against the different types of streptococcal M protein. The vaccine, which has proven to be effective in animal studies, is scheduled to undergo clinical trials in late 1995.

While conventional vaccines are delivered through the bloodstream, Fischetti's works at the mucosal surface—the membranous lining of the digestive, respiratory and reproductive system where 90% of microbial infections begin. Such mucosal vaccines, says Fischetti, "have changed our thinking, and a lot of other people's thinking, about how to develop safe and effective vaccines. If you block the entrance of the pathogen at the mucosal surface, you can circumvent all kinds of complications that occur once the pathogen has entered into the body's tissues."

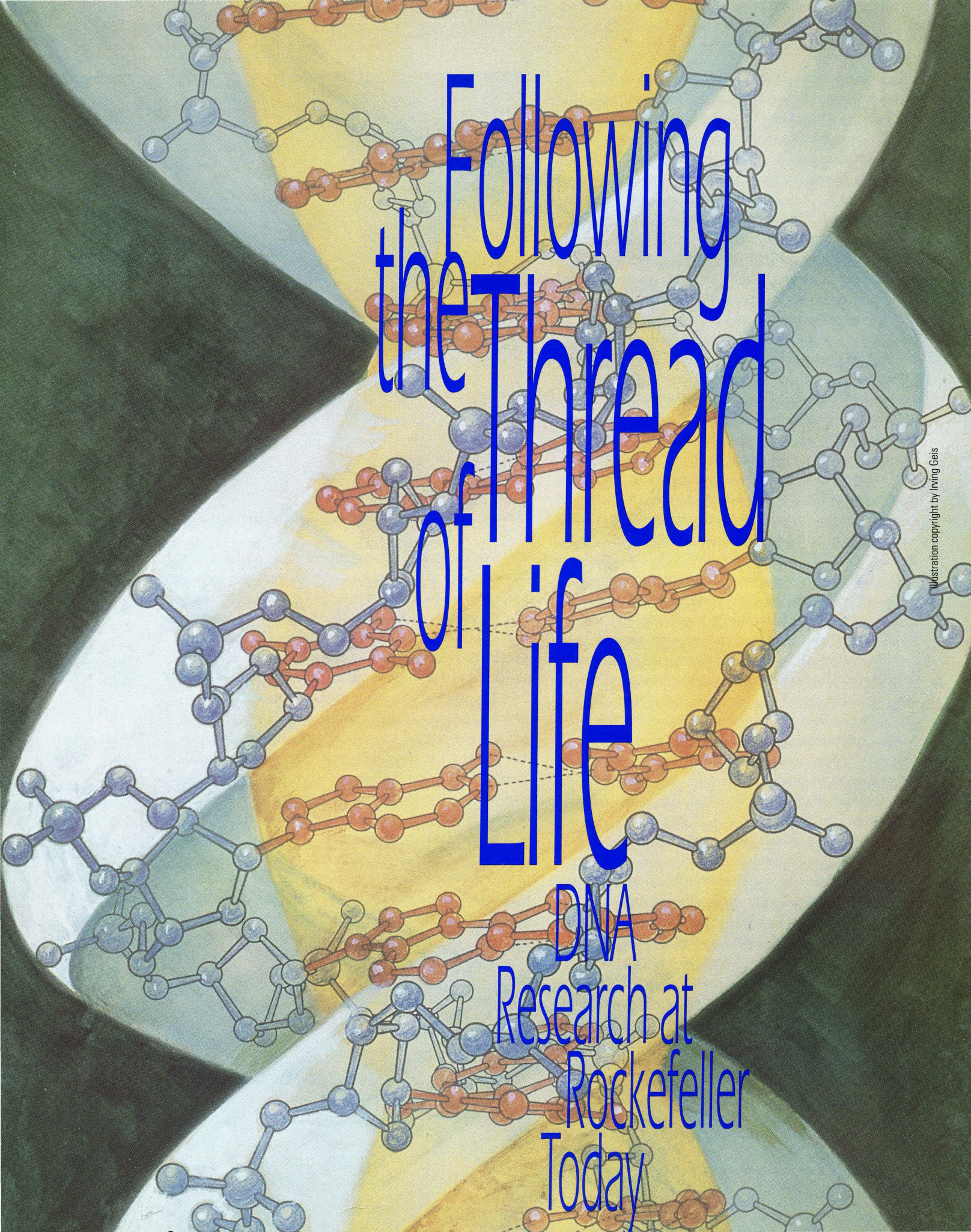
The conserved M protein anchor region identified by Fischetti's group has also led them to identify another possible target for antibiotics, one that would be effective against all strains of strep and other gram-positive bacteria. The attachment of M proteins to the bacterial cell wall requires a specific enzyme; without this enzyme, the bacteria cannot construct its protective M protein shield. Fischetti's lab is working to isolate this enzyme. A substance that would specifically inhibit the function of this essential bacterial enzyme might serve as a novel type of antibiotic.



Above, Dr. Vincent A. Fischetti and his colleagues use recombinant DNA technology to design vaccines applicable to a wide variety of pathogenic microbes.

The conserved anchor region of the M protein can also be genetically fused to the DNA encoding a target protein used by any pathogenic microbe, from pneumococci to the AIDS virus. "You just cut and paste DNA," says Fischetti. Hybrid DNA molecules can then be transferred into a harmless bacteria, where the target protein of the pathogenic microbe will be anchored to this innocuous bacteria's surface. Once introduced into humans, the immune system will generate antibodies against the target protein displayed on the surface of the bacteria, providing immunity against a pathogenic microbe that has never been previously encountered.

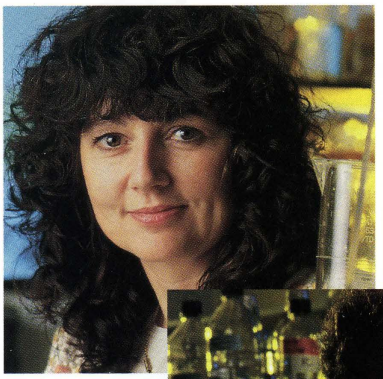
"The investigations carried out by Avery and his school," wrote Dubos, "have provided the pattern, the master plan, used by our generation for the immunochemical study of infectious processes." With today's ability to read the DNA inside an infectious microbe to better understand how it causes disease, and to rewrite DNA to design new drugs and vaccines, it is clear that Avery's master plan for infectious disease research is a legacy that endures. S

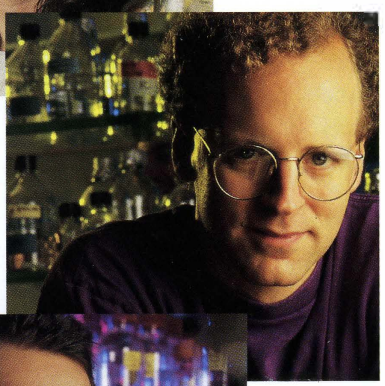


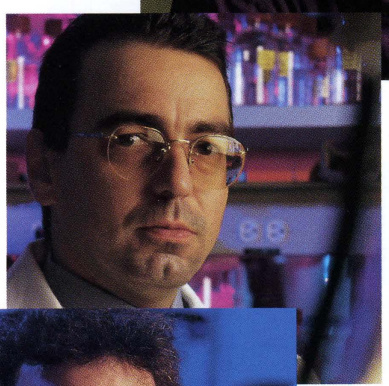
Following the Thread of Life

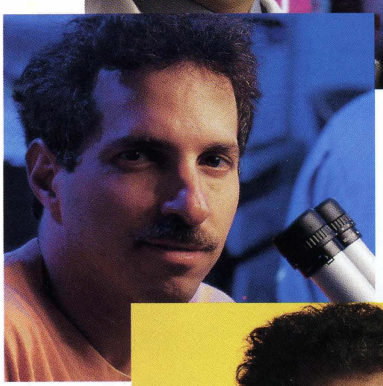
DNA Research at Rockefeller Today

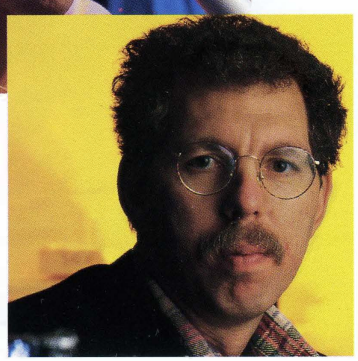
Fifty years ago, Rockefeller scientists Oswald Avery, Colin MacLeod and Maclyn McCarty published the paper that launched a biological revolution. Their research disclosed that DNA is the stuff of genes, its threadlike fibers endowed with the instructions for weaving the intricate tapestry of life.











Researchers around the world have been pursuing the thread of life ever since. In the 1950s, DNA's double-helical structure was unveiled. In the '60s, the genetic code was deciphered and its mode of translation into proteins revealed. In the '70s, the complex nature of genes in higher organisms was discovered. In the '80s, the techniques of genetic engineering were refined. In the '90s, the effort to map humanity's entire genetic endowment began, and the first experiments in gene therapy got under way.

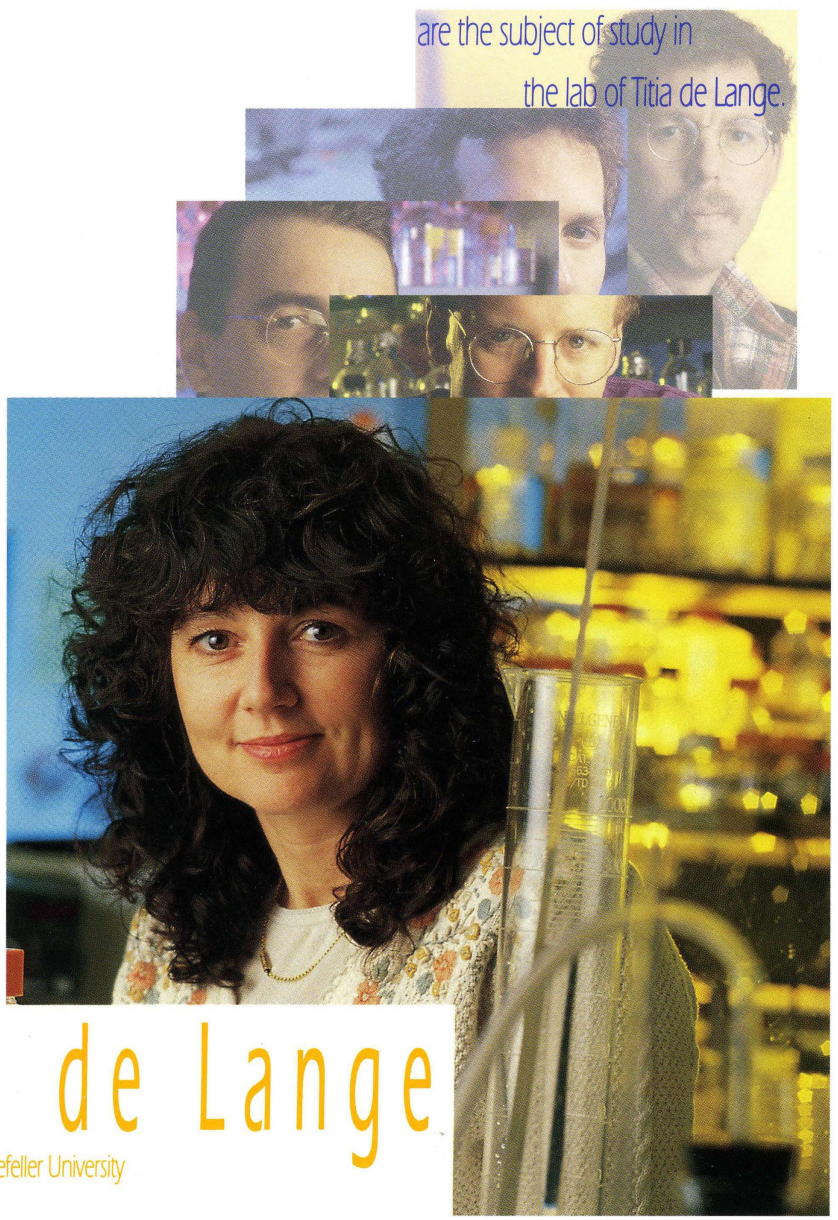
But despite these exciting advances, countless questions remain. How is DNA conserved and protected within the nucleus? What controls its cycles of replication and distribution? How is the readout of genetic information regulated? How do the genes control life's processes, including the development of new life itself? What contributions do genes make to disease, and how can those culprit genes be found?

The answers to these questions — and many more — are being sought by a new generation of researchers at Rockefeller, who continue the legacy of DNA research begun half a century ago. Highlighted here is the work of five of them.

by Susan Blum

To Serve and Protect: The Multiple Functions of Chromosome Ends

Telomeres—protein/DNA complexes that cap chromosome ends—are the subject of study in the lab of Titia de Lange.



Titia de Lange

Associate Professor, The Rockefeller University

After Avery and his colleagues discovered that DNA carries genetic information in bacteria, scientists came to realize that DNA is the stuff of genes in all creatures, including humans. In each living cell, the genes are aligned next to one another on chromosomes. Bacterial cells, which have no nucleus, carry their entire genetic endowment in one circular chromosome. But the genes in the nucleated cells of higher organisms are distributed along multiple linear chromosomes—and therein lie potential problems.

For one thing, the cellular machinery that duplicates linear chromosomes in each cycle of cell division cannot copy their very ends, thus posing the risk that the chromosomes will eventually be whittled right out of existence. For another, if left unprotected, the ends of linear chromosomes are subject to loss through fusion with one another or degradation by cellular enzymes that patrol for dangerous breaks in DNA.

Telomeres—the subject of study in Titia de Lange's lab—apparently solve both these problems. Telomeres are complexes of specialized proteins and repetitive DNA sequences that cap the ends of linear chromosomes. They solve the "end-replication problem" by interacting with an enzyme called telomerase, which adds back the DNA that would otherwise be lost when the chromosome is duplicated. And they solve the problem of potential fusion and degradation by disguising chromosome ends from the cell's enzymatic surveillance mechanisms.

Ironically, the two functions of the telomeric complex are inherently self-contradictory, hiding the DNA from the patrol enzymes while handing it over to the telomerase. Says de Lange, "It's obvious to most people that the resolution

of this contradiction is going to lie in the interactions between telomeric proteins and DNA." But though the DNA of telomeres has been under study for more than fifteen years, very little is yet known about the proteins associated with it.

de Lange and her colleagues are hunting for telomeric proteins in vertebrates, and so far they have found two candidates. One might coat the entire length of the telomere; the other recognizes the repetitive telomeric DNA only at the telomere's very end.

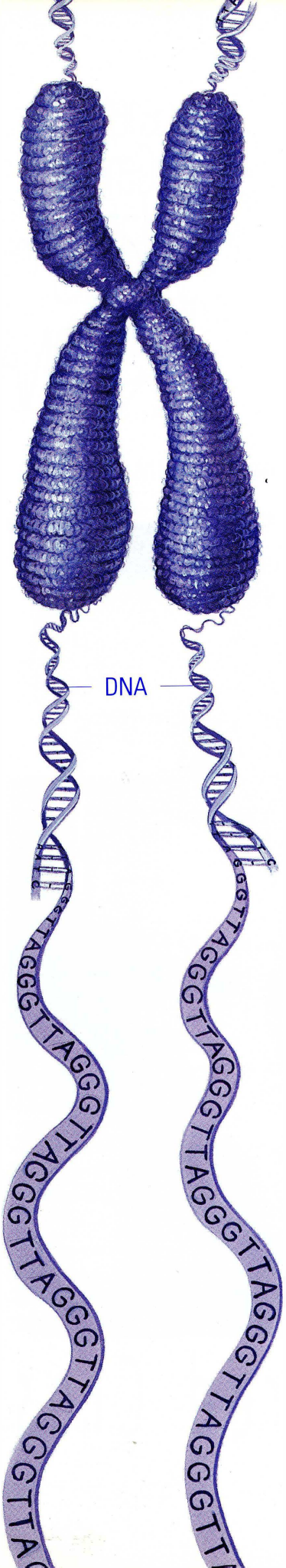
de Lange is investigating whether these proteins play a role in interacting with or regulating telomerase, an enzyme of intense interest to cell biologists. Telomerase is thought to be active in germ-line cells, the progenitors of the sperm and egg cells that transmit genes to the next generation. There, the enzyme ensures that the myriad rounds of replication involved in producing the cells do not shave down their precious genetic inheritance. In most cells of the body, though, telomerase is normally inactive, and the chromosomes are progressively whittled down.

Some scientists believe this shortening may trigger the workings of a cellular clock that ticks off the number of cell divisions and eventually tolls the cell's senescence and death.

But some cells never die. These are the cancer cells that evade mortality and divide prodigiously, perpetuating their deadly genetic mutations. At first (as de Lange discovered while still a postdoc) the chromosomes in cancer cells shorten with each replication cycle, just as in normal cells. But recent studies have shown that at some point cancer cells turn telomerase back on, thus stabilizing the cancer cell's chromosomes and perhaps overriding cellular controls triggered by ever-shortening telomeres.

Researchers are hopeful that drugs against telomerase might be a new weapon in the war against cancer—one that targets cancer cells while leaving healthy cells alone.

But de Lange points out, "We need to get down to basics first. We must understand in detail what telomeres are to a cell and what happens to a cell when it loses them. As long as we don't understand that, it's hard to draw any conclusions about tumor formation, or even about normal aging." Given the many novel discoveries that have emerged from telomere research so far, there may well turn out to be additional surprises found lying at what de Lange calls "the far side of the genome." S



Left, Telomeres are complexes of specialized proteins and repetitive DNA sequences that cap chromosome ends. In human cells, the characteristic DNA repeat consists of six bases: TTAGGG, or thymine-thymine-adenine-guanine-guanine-guanine. (The second strand of the DNA double helix is of course composed of the complementary base partners.) Telomeres are very long; in humans, they extend for thousands of base pairs.

Illustration by Terese Winslow

From Generation to Generation: Regulating the Inheritance of DNA

Fred Cross and his colleagues focus on the cell cycle—the events in which a cell grows, duplicates its chromosomes and divides.

Fred Cross

Associate Professor, The Rockefeller University

Perhaps as good a definition of life as any is that life is order. To perpetuate that order, the information carried in DNA must be faithfully transmitted to each new generation of cells—be they the somatic cells that make up most of the body, or the specialized sperm and egg cells that pass chromosomes on to the next generation.

The process by which this order is maintained is the cell cycle—the tightly regulated sequence of events in which a cell grows, duplicates its chromosomes, and bequeaths them to the "daughter" cells into which it divides. In the past decade, research in a wide range of cells—from those of yeast to those of vertebrates—has been converging into a single, highly unified picture of how the cell cycle works in eucaryotic cells, or cells with a nucleus.

Remarkably enough, much of this progress has occurred through what Fred Cross calls "a crazy update" of the experiments conducted by Avery and his colleagues half a century ago. In this modern version, genes are not transferred between different strains of the same species (as Avery did with pneumococci, for instance) to elicit new traits in the recipient cells. Rather, genes are transferred between cells of species as different as yeast and humans, where they are found to perform exactly the same functions in each. The reason: The genes are so essential to the workings of eucaryotes that they have been conserved virtually unchanged across billions of years of evolution.

The genes thus identified code for proteins that play vital roles in cell cycle control. Many events occur in this cycle, which is generally divided into four phases. In the first phase, G1 (for "gap 1"), the cell grows and, at a critical point, commits to reproducing its DNA.

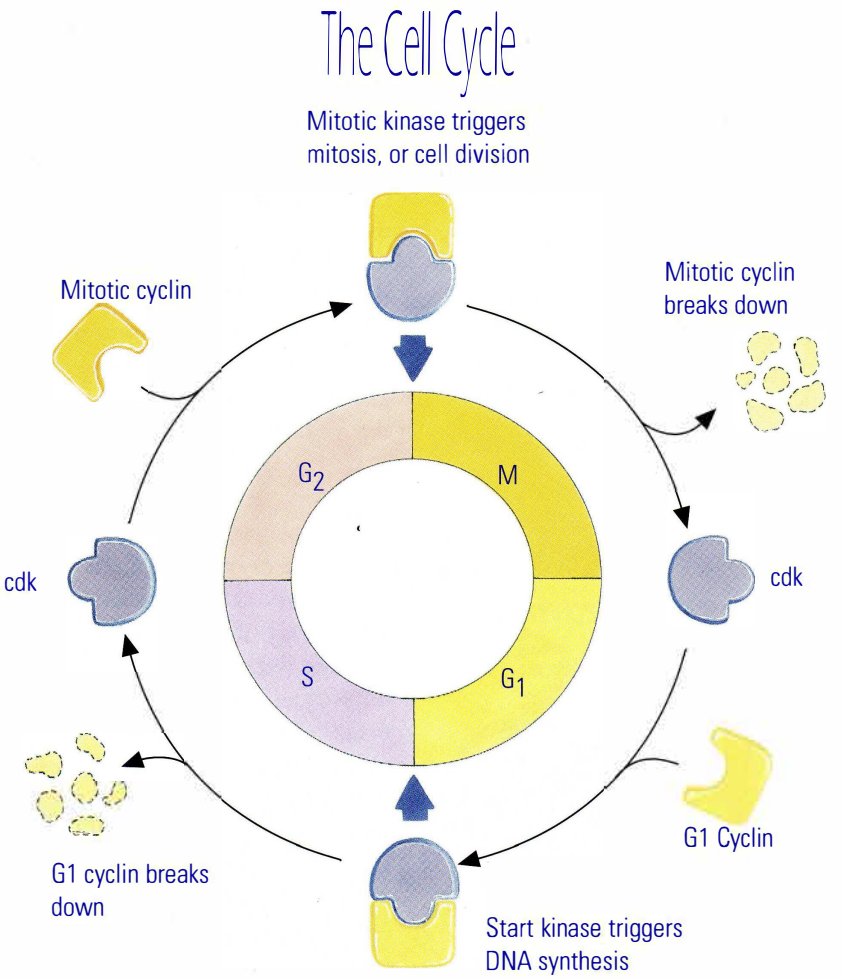
Once this starting line is crossed, a slew of events occurs in the next phase, called S (for "synthesis")—including the replication of chromosomes. The second gap phase (G2) follows, during which the cell makes another commitment—this time, to divide in two. After this critical point, the cell enters the M phase, named for mitosis—the division of the cell's nucleus that is one of the last steps leading up to cell division.

The first insights into control of the yeast and vertebrate cell cycle came from studies of the transition from the G2 to M phase. Experiments disclosed how a particular enzyme, a type known as a kinase, serves as the master regulator that triggers mitosis. This molecule is actually composed of two subunits. The catalytic part, called a cdk, is present in constant amounts throughout the cell cycle. But the kinase can act only when teamed up with another subunit, called a cyclin, whose abundance varies at different times in the cycle.

Later experiments showed that the same basic scenario works to regulate events in the transition between the Gl to S phase, when cdk molecules team up with cyclins (different from those involved in the M phase) to orchestrate the events culminating in chromosome replication. This cell cycle stage is the focus of work in Cross' lab, where he and his colleagues are studying the function and regulation of genes coding for a family of yeast G1 cyclins called CLNs.

The cell cycle story has taken on even greater complexity with the discovery of proteins in both yeast and humans that inhibit the activity of cdk/cyclin complexes. The first protein ever to be identified as an inhibitor—called FAR l—is a yeast protein also under study in the Cross lab. The Rockefeller researchers' recent discovery that levels of FAR 1 rise and fall cyclically will help further refine current models of the intricate networks controlling the cell cycle.

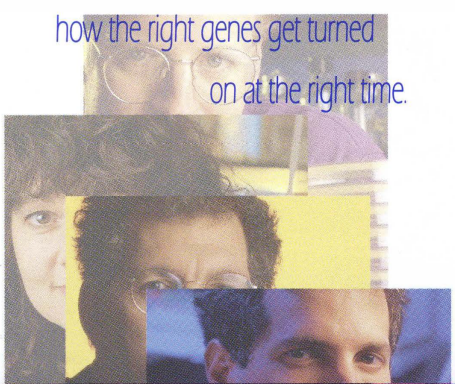
Today's cell cycle studies may one day lead to advances in understanding and treating cancer, a disease of unrestrained cell growth and division. Says Cross, "It always made sense that links would exist between cell cycle control and cancer, but only in the past year or so have those links become real, rather than speculative." For instance, one inhibitory protein has been tied to cellular pathways regulated by a tumor-suppressor gene called p53. Researchers have also learned that the deregulation of certain cyclin molecules can help transform a normal cell into a cancerous one. With an ever-increasing understanding of the intricately choreographed steps regulating the cell cycle, it may someday be possible to stop the music when the molecular dancers start spinning wildly out of control. S



Above, The cell cycle has four phases: Gl (for "gap l"); S (for "synthesis" of DNA); G2 (for "gap 2"), and M (for "mitosis," or cell division). Master molecules called kinases trigger the two main events of the cell cycle—the duplication of DNA and the division of the cell into two progeny. The kinases are composed of two subunits. The catalytic parts, called cdks, are present in constant amounts throughout the cycle. But the kinases can act only when teamed up with other subunits, called cyclins, whose abundance varies at different times in the cycle.
Illustration by Terese Winslow

Required Reading: Molecular Machines Control Cells' DNA Readout

In the lab of Stephen Burley, scientists are envisioning how the right genes get turned on at the right time.



When the seminal paper by Avery *et al.* was published, DNA—with its mere four chemical subunits—was widely believed too "stupid" a molecule to carry all the information required to construct and maintain even the simplest life forms. But in the two decades that followed, the wisdom of DNA was revealed, as researchers deciphered its code and showed how genes serve as the blueprints for all the proteins an organism requires.

In complex multicellular organisms, every cell possesses a full complement of genes, but turns only some of them on. Each different cell type thus produces a different array of proteins. And, as Stephen Burley explains, it is these differences in protein content that make "a liver cell resolutely a liver cell, a skin cell resolutely a skin cell."

Genes are turned on in transcription, the process by which DNA is read out into a closely related molecular intermediate called messenger RNA. (In a later step, the instructions in messenger RNA are then translated into protein.) Transcription is accomplished by complex molecular machines that forge intricate relationships between DNA, a copying enzyme called polymerase, and transcription factors that interact with the DNA, the polymerase, or both. Some transcription factors serve as molecular bookends, positioning the copying enzyme at the proper place to start a gene's readout. Other factors act as accelerators or brakes, controlling the speed of transcription and ensuring that only certain genes are read out in certain cells.

Burley and his colleagues explore the workings of these molecular machines on an atom-by-atom basis, using the technique called x-ray crystallography. Their studies of DNA/protein interactions have disclosed a submolecular world of astonishing beauty and variety. For instance, they have shown that TBP, a positioning factor, sits astride DNA like a saddle, while HNF-3, an activating factor, envelopes DNA with gossamer butterfly wings.

Just as the work of Avery and his colleagues revealed that DNA was not as stupid as once believed, so current explorations disclose that DNA is not as static as the famous double-helical structure used to imply. In fact, crystallographic studies show that DNA may undergo dramatic conformational changes when in the grip of the transcriptional machinery. For example, Burley and his colleagues found that when the TBP saddle drops over DNA, the DNA twists more than 100 degrees from its normal orientation. Likewise, they showed that some activating and repressing factors cause DNA to bend and loop, bringing hitherto-distant gene regions into close proximity.

Transcription factors can also

Stephen Burley

Professor, The Rockefeller University

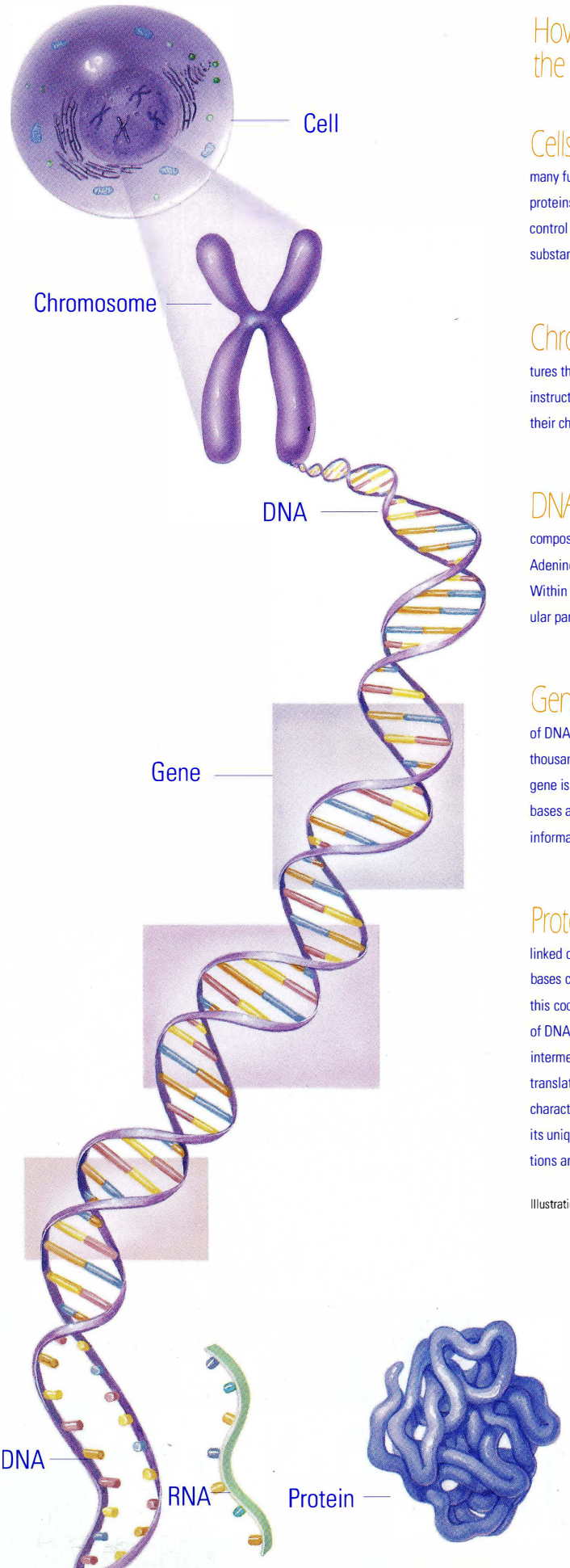
Investigator, the Howard Hughes Medical Institute

affect DNA's higher organization. Normally, DNA is spooled around proteins called histones. Such packaging corrals the mind-boggling six feet of DNA found in each cell, but it also hinders transcription by hiding essential gene-control regions. (Indeed, many scientists believe that such hindrance by histones is a vital component of transcriptional control, not merely a troublesome by-product of a cell's DNA packaging needs.) Now, recent studies by Burley and others are showing that transcription factors can affect DNA/histone interactions in ways that alter DNA's usual patterns of nesting within the nucleus.

Says Burley, "It is becoming clear that to know how the right gene gets transcribed at the right time, we will have to understand not only protein/protein and protein/DNA interactions, but the much larger questions of DNA structure and organization, as well."

The more scientists learn about the machinery that controls gene activity, the better their chances of eventually tinkering with those machines for their own therapeutic purposes.

For instance, part of the process that turns a cell cancerous involves the deregulation of transcriptional controls. By throwing molecular monkey wrenches into transcriptional machines gone amok, scientists may someday be able to slow or even halt the progression of cancer. They may also be able to construct their own transcription machines from scratch, to safely and accurately regulate foreign genes delivered in gene therapy for a wide range of diseases. The ultimate goal, says Burley, is to enlist knowledge gained from crystallographic studies "not only to understand transcription, but actually to control it in the context of the human body." S



How DNA Weaves the Fabric of Life

Cells Cells contain many structures and perform many functions crucial for life. Their workhorses are proteins, the molecules that shore up a cell's structure, control its chemical reactions, and determine which substances enter and leave.

Chromosomes Chromosomes are the structures that package DNA, the substance encoding the instructions for proteins. Some organisms sequester their chromosomes within a cell nucleus; others do not.

DNA DNA is a double-stranded helical molecule composed of four different subunits, or bases — Adenine (A), Thymine (T), Guanine (G) and Cytosine (C). Within the helix, the bases always link up with a particular partner: A pairs only with T, G only with C.

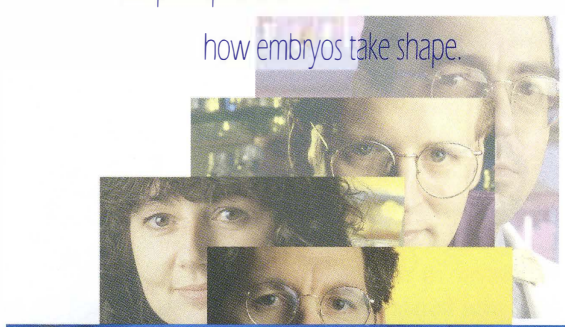
Genes A gene is an information-carrying segment of DNA. Genes can be thousands or even hundreds of thousands of base pairs long. The information in each gene is different, depending on the order in which the bases are arrayed in the gene. Most genes contain the information for constructing proteins.

Proteins Proteins are long chains of amino acids linked one to another. Different sequences of DNA bases code for different amino acids. The cell reads out this code in a series of intricate steps. First, one strand of DNA is transcribed into a closely related molecular intermediate called RNA. Then the cellular machinery translates RNA into proteins. The unique order of bases characteristic of each individual gene gives each protein its unique identity. Proteins serve many different functions and give cells their essential characteristics.

Illustration by Terese Winslow

A Matter of Choice: Determining Cell Fate in Embryogenesis

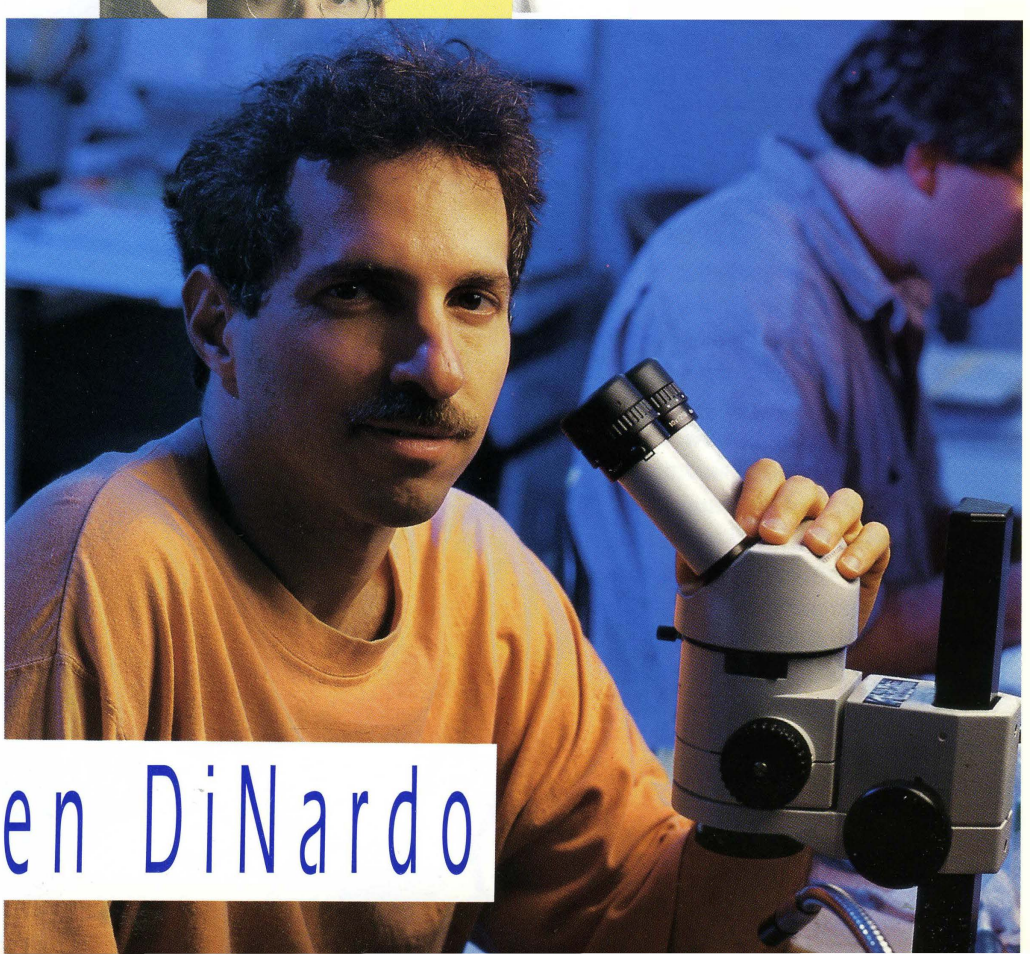
Stephen DiNardo and co-workers are pursuing the principles that control how embryos take shape.



The genes that get read out into proteins do more than maintain an organism's everyday life. They also control the emergence of new life itself, in what Stephen DiNardo calls "the mystique and the magic" of embryologic development.

Development consists of a series of choices. The original fertilized egg has the potential to become every type of cell the mature organism will require. But with each round of successive cell division, the options for each daughter cell grow progressively limited, as the cells make choices that nudge them closer to their final identity. These choices are made manifest in the emergence of cellular patterns of ever-increasing complexity within the embryo. At each step, the decisions are dictated by positional information that tells a cell where it is in relation to other cells in the emerging cellular pattern.

Researchers in the DiNardo lab are investigating how positional



Stephen DiNardo

Associate Professor, The Rockefeller University

information specifies the ten or so different epidermal cell types in the fruit fly embryo. Each differentiated cell type has a different shape and boasts a particular surface appearance. Some cells are smooth, while others sport a little hair, or denticle, of a characteristic length, thickness and orientation.

While a fly's prickly surface might not seem very aesthetic, it is yielding elegant information about embryonic choices—including some made in the earliest stages of development.

During that early period, positional information is conveyed to a cell from "organizing centers," areas that serve as sources of signals directing the differentiation of large numbers of cells into many different cell types.

Scientists have known about organizing centers since the 1930s, but the search for the signals they send has long been fruitless. Recently, however, researchers from a number of labs identified the first such signaling molecules in vertebrates, and found that they all belonged to the quirkily named "hedgehog" family of proteins.

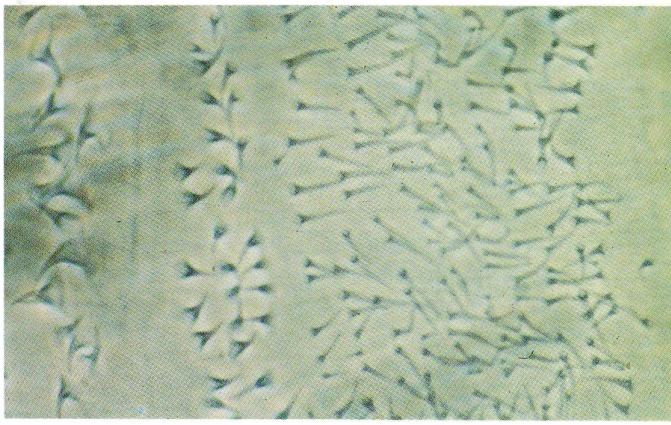
Though these discoveries were loudly trumpeted in the press, the initial identification of the hedgehog gene, and of the crucial role it plays in development, was actually accomplished in the mid-1980s in work done in the fruit fly. That hedgehog genes are proving important in so many different species comes as no surprise to biologists, who keep finding that the fundamental principles—and molecules—controlling development are the same in all animals.

Many significant findings about hedgehog proteins in fruit flies are being made in DiNardo's lab, where he and his colleagues are studying the role of hedgehog and another protein called wingless. Their research has disclosed a two-phase process of interactions between cells that express either hedgehog or wingless. In the first phase, these cells signal each other over short distances to stabilize hedgehog and wingless production. This stabilization creates two organizing centers that serve as continuous sources of hedgehog and wingless proteins, which then act over a distance to specify the fate of several different epidermal cell types.

DiNardo believes that this two-phase model will prove to be a general one, not only in flies but in vertebrates, too. But while the overall plot line may stay the same, the players will vary. In both flies and vertebrates, although a limited number of conserved families of signaling molecules keeps turning up (including hedgehog-type molecules and wingless-type molecules) they interact with each other in various combinations.

DiNardo is not disconcerted by these variations. "There will be perhaps three or four different ways in which molecules like hedgehog can act, and nature will use them at different times and in different places in the development of different organisms," he says.

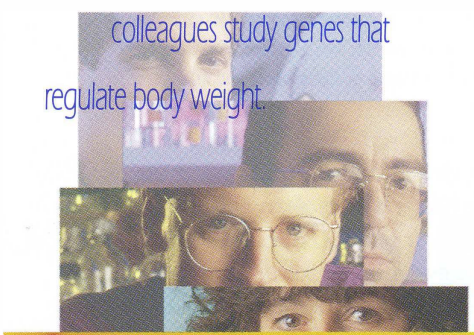
Insights like these would not have been possible without the revolution begun by Avery and his colleagues. Before then, developmental biologists were limited to observing the effects of positional information by moving various parts of tissue around in the embryos of animals such as chicks and frogs. Today, researchers can also use the powerful tools of genetics in invertebrates such as flies and worms, and then analyze the function of those genes directly in virtually any species, using the techniques of molecular biology. As their studies continue to disclose the essential mysteries of development, and the fundamental similarities of this process in all organisms, it is clear why DiNardo says, "This is really a very exciting time." S



Above, Each different cell type in a fruit fly's epidermis has a different shape and appearance. Some are smooth, while others sport little hairs of various lengths, thicknesses and orientations.

Gene Hunting: The Search for the Genetic Roots of Disease

Jeffrey Friedman and colleagues study genes that regulate body weight.



Half a century has passed since Avery and his colleagues showed that the secret of life lies in DNA. But until very recently, most of those secrets lay tantalizingly out of reach, due to a lack of lures and hooks with which to fish out individual genes from the ocean of DNA possessed by each organism.

Within the past decade, however, molecular biology has provided the tools that let scientists identify and analyze individual genes of interest. As the newspaper headlines attest, some of the genes of greatest interest are those that, singly or in combination, cause or contribute to the many diseases that plague mankind. Among those diseases is obesity, a condition associated with such potentially life-threatening health problems as diabetes and high blood pressure. Recently, Jeffrey Friedman and his colleagues took a major step toward identifying one of the genetic contributors to obesity by cloning—or pulling out from the chromosomes—a gene, called ob, that may play a central role in weight control.

Studies have shown that from 60 to 90 percent of the differences in people's weights is due to their genes. For many years, researchers had hypothesized that these genes play roles in a complex feedback system that maintains body weight at a particular set point. Such a system would involve signals from points in the body's periphery (the fat cells, for instance) that reach receptors in a brain region called the hypothalamus (and perhaps elsewhere, too) to report on how much the body currently weighs and how well it has recently been nourished. The brain in turn would regulate various responses—such as energy expenditure and food intake—to keep body weight steady. In such a complex system, problems anywhere along the pathway—a faulty signal, for instance, or a defective receptor—could lead to obesity.

To clone the genes involved in this system, researchers turned to the laboratory mouse, a genetically well-understood creature in whom at least five different genes are known to cause obesity in various strains. Friedman and his colleagues set their sites on finding two of these genes: ob (or obesity), which previous studies had indicated might code for the signaling molecule, and db (or diabetes), which appeared to code for its receptor.

By employing a method called "positional cloning," Friedman and his colleagues recently cloned the mouse ob gene. (See "How to Find a Disease-causing Gene," *right*). Subsequent analysis of the gene has

Jeffrey Friedman

Associate Professor, The Rockefeller University

Associate Investigator, the Howard Hughes Medical Institute

provided much evidence suggesting that ob may indeed code for a signaling molecule, produced by fat cells, that sends a message to the hypothalamus to reduce food intake and/or boost energy expenditure once the fat cells have reached a certain mass. Experiments to confirm ob's signaling role are now under way in Friedman's lab, as are studies to positionally clone the db gene.

Friedman and his colleagues have found that the ob gene is conserved, or similar, in many vertebrates from eels to humans, whose ob gene shares an 84 percent similarity to that of the mouse. Such conservation suggests that a feedback system for weight maintenance has been in place for eons, and that the genes controlling this system have been subjected to intense selective pressure.

"Humans evolved in an environment where getting enough calories was difficult, so selection might have favored versions of the genes that allow us to deposit food efficiently as fat," Friedman says. Once highly adaptive, these versions of the genes would now be maladaptive in environments such as the United States, where sufficient calories are available to many—and where the rate of obesity has soared to 30 percent.

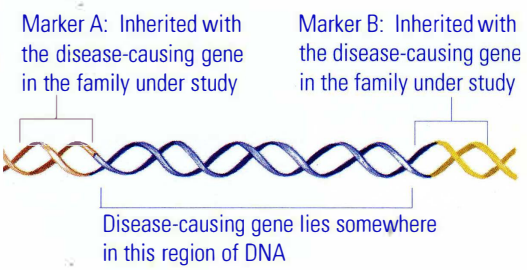
Some of the strongest evidence in support of evolutionary pressure on weight maintenance genes comes from studies of aboriginal populations such as the Pacific Islanders of Micronesia, who now have extremely high rates of obesity and diabetes. "In general, the more severe the environmental conditions in past history, the more profound the obesity in modern times," Friedman says. Studies of such populations offer extraordinary opportunities to answer the fundamental question about genes like ob, to wit: Is their role in human obesity as clear as their role in the rodent form of the disease? Friedman and his colleagues are now collaborating with residents of the Micronesian island of Kosrae to answer this question.

The confirmation of ob's role in human weight maintenance would open up many exciting possibilities for a "more rational approach for devising novel therapies for weight disorders," Friedman says. Such therapies could be used not just to lower weight in the obese, but also to boost it in those who are profoundly underweight due to conditions such as cancer and AIDS. S

How to Find a Disease-causing Gene

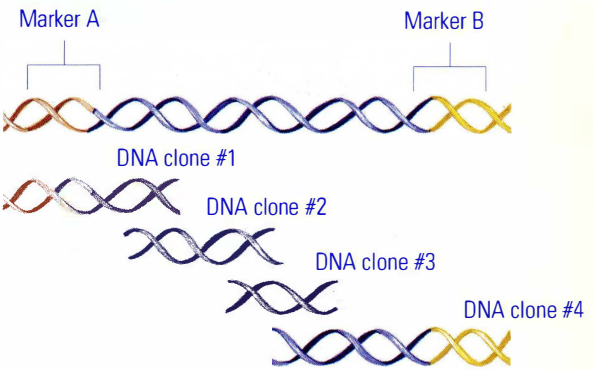
1. Find DNA Markers

Markers are DNA sequences that lie close to a particular gene and are inherited with it. By analyzing how various DNA markers are inherited among members of a family in which the disease sometimes occurs, scientists can identify markers flanking the disease-causing version of the gene. This pinpoints the DNA region in which to look for the gene itself.



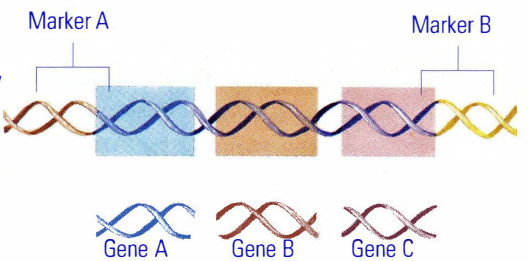
2. Assemble a Physical Map

A physical map tells scientists how much DNA lies in the region between the markers, and makes further molecular analysis of the DNA possible. To create the map, DNA in different parts of the region is cloned, or reproduced, in vectors such as bacteria or yeast. These cloned DNA segments can then be aligned by identifying regions of overlap.



3. Identify All the Genes

More than one gene may lie in the region, and scientists must identify all of them before homing in on the one causing disease. Only some of the DNA in the region makes up genes; the rest is DNA with no known function. The genes are identified using various biochemical tricks to separate the genes from the rest of the DNA.



4. Sequence and Compare Genes

All DNA is made up of just four bases, or subunits. Each gene differs from all others in the particular sequence in which those bases appear. The base sequence for each gene in the region can be determined, and then compared with the sequence of the same gene as it exists in people without the disease. Mutations in a gene from a person with the disease identify that gene as the culprit.

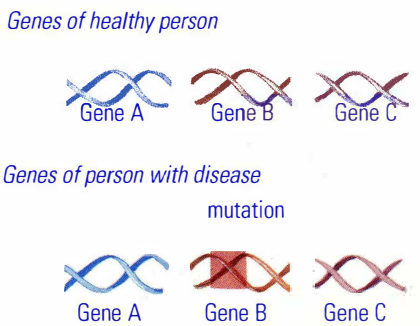
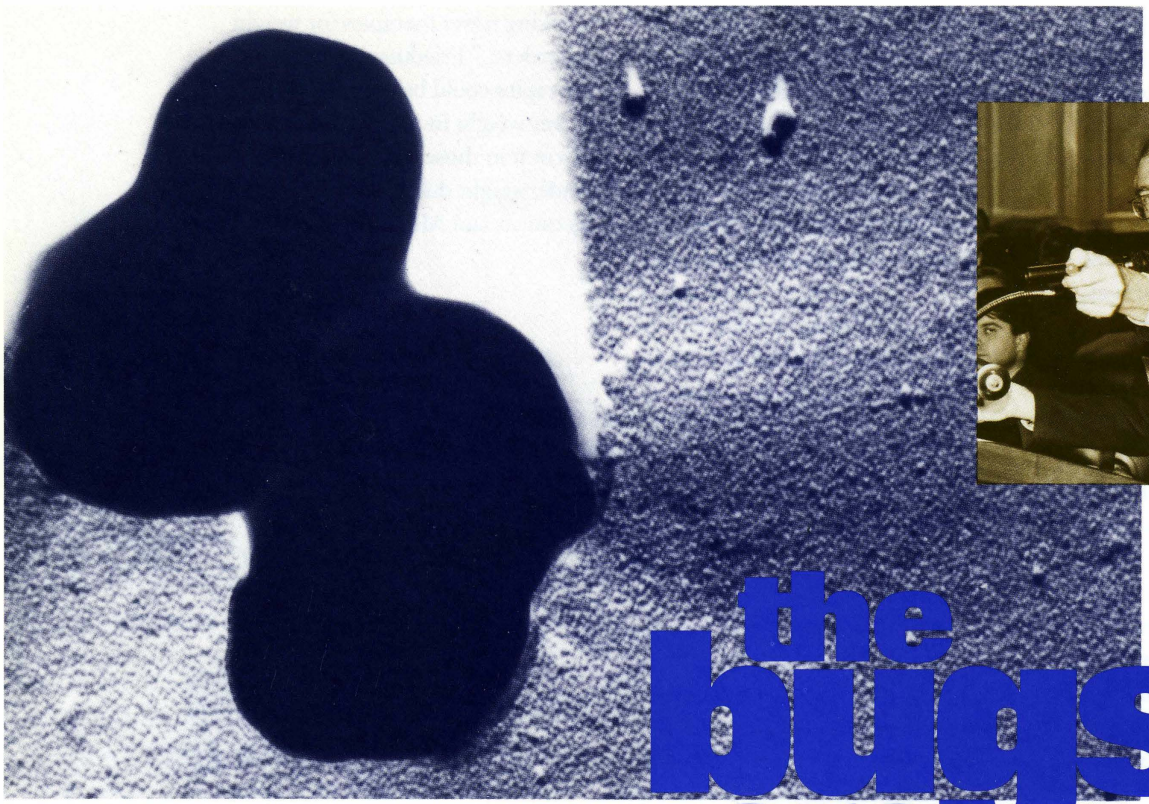
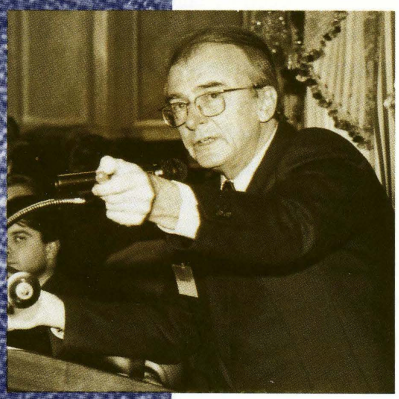


Illustration by Terese Winslow





the bugs are back

Sounding the Alarm on Antibiotic-resistant Bacteria

by Doron Weber

During the past four to five years, medical practitioners, microbiologists and public health professionals all over the world have begun to voice concern over the alarming increase in the number of disease-causing microbes that have become resistant to drugs that once checked them.

"Antibiotic-resistant bacteria are already involved in the spread of hospital-acquired diseases such as pneumonia, bloodstream and wound infections, and in community-acquired diseases such as tuberculosis, meningitis, lung and middle-ear infections across America," says Professor Alexander Tomasz, head of Rockefeller University's laboratory of microbiology and a world-known authority on bacterial antibiotic resistance.

"As more and more common bacteria acquire resistance genes, many of the diseases we thought we had under control are coming back. We are looking at a problem with the potential for causing a public health crisis."

Already, estimates of the annual cost of antibiotic resistance in the United States range as high as $30 billion. Tomasz believes it is imperative for basic researchers and public policy makers to join ranks now, before the cost in dollars is dwarfed by the rising toll in human lives.

Spreading Multiresistance

Tuberculosis, once thought to be a plague of the past, returned during the 1980s armed with resistance to several previously effective antibiotics. By 1992, strains of tuberculosis appeared that were resistant to most of the 12 potentially usable drugs in existence to treat it.

Tuberculosis is only the most well publicized of the returning, newly resistant plagues. In 1992, over 19,000 patients died in the U.S. of various bacterial infections acquired while in the hospital; and a large proportion of the microbes that most frequently cause these infections have become resistant to antibiotics, according to the Centers for Disease Control. The

Above, Strand of DNA entering a pneumococcal cell. *Inset,* Rockefeller Professor Alexander Tomasz points out the dangers of antibiotic-resistant bacteria at a lecture organized by the Public Health Research Institute and sponsored by Lederle Laboratories.

available data indicate that this problem will only get worse.

For example, almost half of all the hospital isolates of the bacterium *Staphylococcus aureus* have become resistant to the most useful penicillin-type antibiotics. This bacterium is the most frequent cause of wound, lung and bloodstream infections acquired in the hospital; and there are many strains of staph against which there now remains only a single effective antibiotic, called vancomycin. Moreover, the genes for resistance, which reside in bacterial structures or loops of DNA called plasmids, can be passed on not just to their progeny but also to bacteria of different species.

A similar scenario is emerging among pneumococci, one of the most frequent causes of community-acquired diseases, particularly in small children. Pneumococcus is currently responsible for tens of thousands of bloodstream infections, 500,000 new cases of pneumonia and 6 million new cases of middle-ear infections each year in the United States.

"We now have several case descriptions from the U.S. of infants with meningitis caused by resistant pneumococci who are no longer responding to therapy by penicillins and cephalosporins," says Tomasz. As a result, doctors have had to use vancomycin, the same drug that has become a "last resort" antibiotic against staphylococci as well. In at least one of these cases, the meningeal disease left the infant neurologically devastated.

To make matters even worse, the genes providing bacteria with resistance to vancomycin are already present in yet another hospital-borne pathogen called *Enterococcus faecium*. Dr. Sandra Handwerger, clinical scholar and assistant professor in the Tomasz lab—and one of the first to identify the biochemical basis of vancomycin resistance—shares the worry of most biochemists that these resistance genes will find their way into the already multiresistant and highly virulent strains of staphylococci and pneumococci, posing a potentially alarming scenario for hospital patients. If vancomycin fails, there is no drug currently available to stop these bacteria. "Many people," Tomasz argues, "feel that such an event will bring us close to a post-antibiotic era."

Post-Antibiotic Era

For a brief, heady period after Alexander Fleming first found in 1928 that a colony of mold in his lab dish exuded a substance, called penicillin, that killed bacteria, penicillin and its descendants became miracle drugs. But man's victory over microbes proved short-lived. While most bacteria will succumb to a powerful new antibiotic, a few have genes, or genetic mutations, that enable them to resist the antibiotic attack, and these produce resistant descendants. So only the fittest—that is, the most resistant—bugs survive. Each surviving bacterium can leave over 16 million offspring within 24 hours.

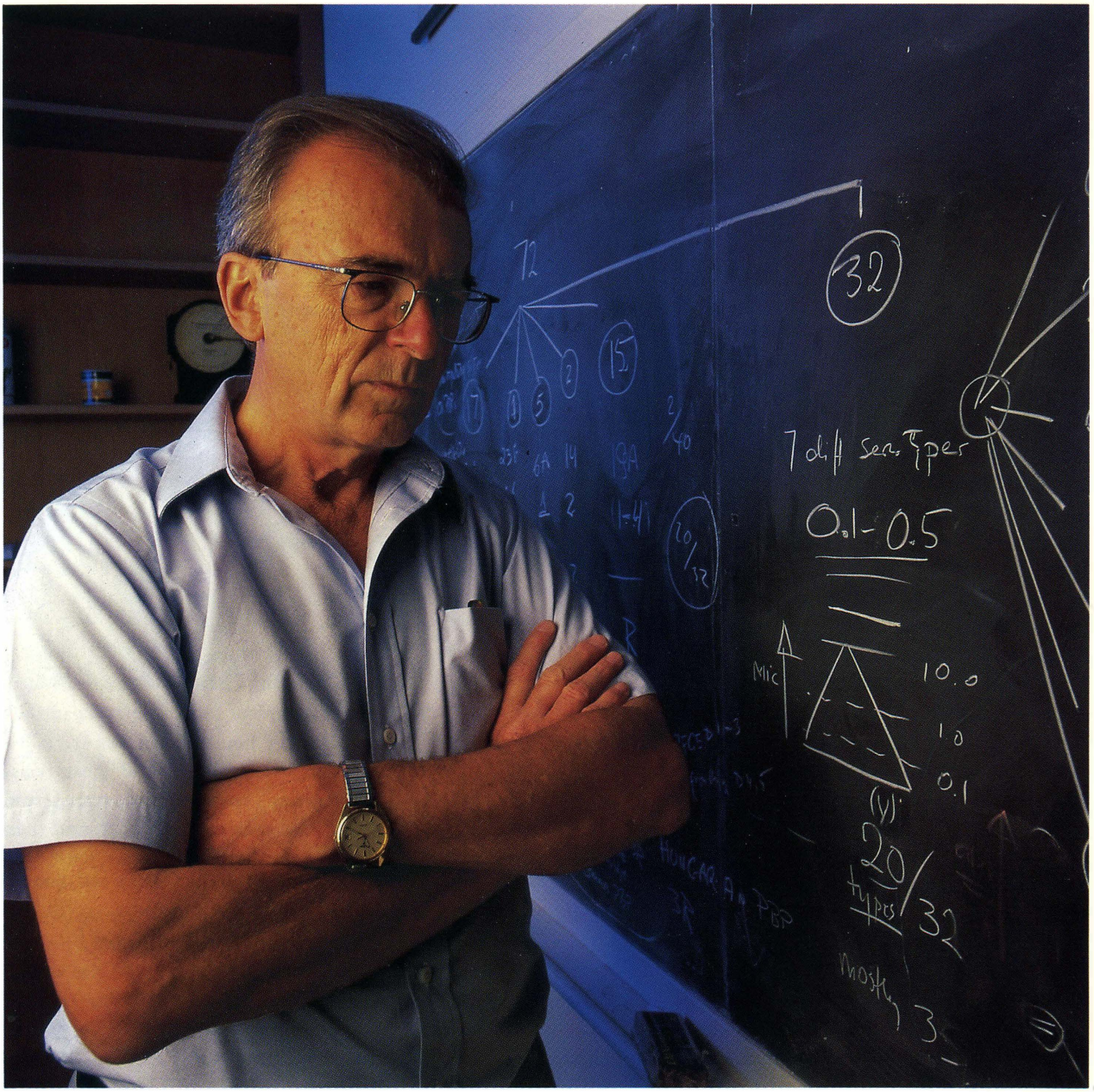
"On an evolutionary scale, bacteria and host have been facing each other and coexisting for billions of years" explains Tomasz. "The discovery of antibiotics introduced a radically new element into this face-off. The devastating effectiveness of penicillins and other drugs introduced during the 1940s forced the microbial world to mobilize its genetic resources. Today, after only 50 years—an incredibly short span on the evolutionary time scale—bacteria have developed a remarkable variety of mechanisms to resist every known usable antibiotic."

" Today, after only 50 years—an incredibly short span on the evolutionary time scale—bacteria have developed a remarkable variety of mechanisms to resist every known usable antibiotic."

The most dangerous are bacteria that seem to be able to collect many mechanisms of resistance and can thus gradually become resistant to all antibacterial agents. And these resistant genes can be spread, transferred from one species to another, and then jet from one part of the world to another.

"Drug-resistant bacteria can spread geographically," says Tomasz. "The rule seems to be that wherever a resistant strain is reported in one part of the world, as time goes by it will find its way to other parts of the world." For example, Tomasz's lab, a world leader in molecular epidemiology (a method that can identify the offspring of resistant bacteria through DNA-level fingerprints), helped track the movement of multiply resistant clones of pneumococci: One was tracked between Iceland and Spain where vacationing Icelanders may have unknowingly picked it up. Another clone, most likely originating in Spain, was tracked literally all around the globe—to Portugal, France, Croatia, South Korea—and this pneumococcal clone has now spread widely across hospitals and day-care centers in the U.S.; the carriers may have been unsuspecting travelers.

Travel, tourism and migration have made antibiotic resistance a potential worldwide threat to public health. Tomasz argues that we have to "rethink and renegotiate" our whole relationship to the procaryotic world.



Above, Tomasz ponders a puzzling epidemiological triangle—a high degree of antibiotic resistance goes with a low degree of genetic diversity among the bacterial isolates—as he stands before a blackboard in his laboratory.

Alex Tomasz: A Lifelong Scientific Commitment

Born in Hungary, Tomasz was studying biology and chemistry at the University of Budapest when he was forced to flee the country after the Soviets cracked down on the Hungarian uprising in 1956. He arrived in New York penniless, and took a job as a technician at Sloan-Kettering Institute before earning a Ph.D. in biochemistry at Columbia University. Upon graduation, Tomasz, who was interested in molecular genetics and the surface of bacterial cells, decided to focus on how pneumococci manage to capture and internalize DNA molecules during the process known as genetic transformation.

Pneumococcus has the capacity, rare among bacteria, to take up free-floating strands of DNA from its environment. It was this capacity, dubbed "competence," that enabled the Avery lab to identify DNA as the carrier of genetic information in its landmark 1944 paper. Well over a decade later, Tomasz knew there was still only one place to study this phenomenon: The Rockefeller University laboratory of Rollin Hotchkiss, whose group had made enormous advances in elaborating on the discovery of Avery,

MacLeod and McCarty.

After joining the Hotchkiss lab, Tomasz began to study how the pneumococcus bacterium can recognize a DNA molecule in its vicinity. Through what signals does this recognition take place? Among his major findings, Tomasz discovered that competent cells of the pneumococcus release a protein he called "activator" that could induce the uptake of DNA in cells that were not yet competent. The discovery of the activator, the first bacterial hormone, and the subsequent studies on the DNA-binding transport system were, in a sense, a completion of Dr. Avery's discovery of transformation. Over the following years, studies in the Tomasz lab have contributed richly to a wide range of topics in microbiology, such as the chemistry and biological activities of bacterial cell walls and the mode of action of penicillin; they have identified the molecular basis of beta lactam resistance in pneumococci and staphylococci; and discovered antibiotic tolerance or the ability of bacteria to survive antibiotic treatment despite being neutralized. Most recently, the lab's efforts have led to the elucidation of a large number of auxiliary genes needed for antibiotic resistance, genes that may be new targets for pharmaceutical drugs. The Tomasz lab also pioneered in the use of molecular "fingerprints" for tracking the movement of drug-resistant bacteria in hospitals in the U.S. and Europe.

In July 1993, Tomasz organized a one-day workshop at Rockefeller that brought together some of the nation's leading infectious disease experts including those from the CDC, the FDA and the NIH, as well as the presidents of several scientific and medical organizations.

Getting the Message Out: A Busy Year

While his lab continues to study basic problems in microbiology, Tomasz has begun to organize a public response to the emergence of disease-causing bacteria.

In July 1993, Tomasz organized a one-day workshop at Rockefeller that brought together some of the nation's leading infectious disease experts including those from the CDC, the FDA and the NIH, as well as the presidents of several scientific and medical organizations. They focused on the accelerating spread of pathogenic bacteria resistant to antibiotics and asked whether multiresistant bacteria posed a threat to public health in the United States. The resounding answer, they all agreed, was "yes."

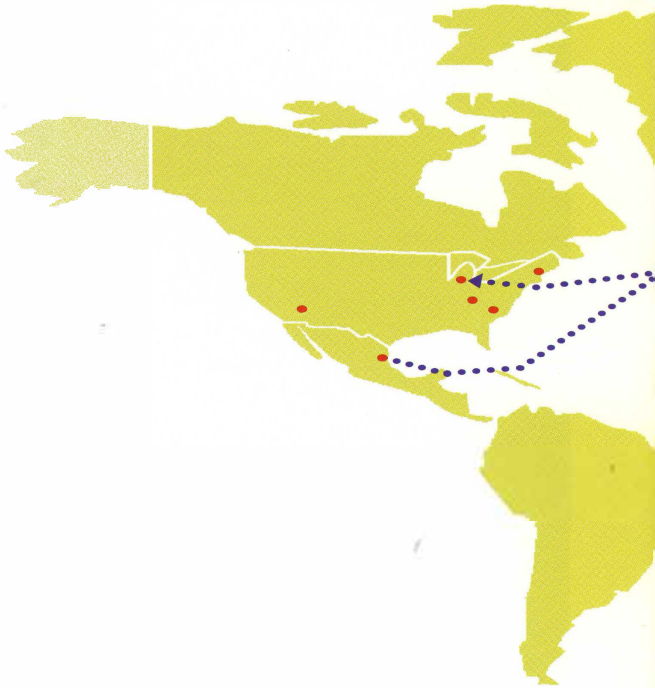
Nine months later, the April 1994 issue of the *New England Journal of Medicine* published the proceedings of the workshop in a special report entitled "Multiple-Antibiotic-Resistant Pathogenic Bacteria" that was authored by Tomasz. Among its recommendations, the report called for greater awareness of the problem among clinical microbiologists, government health authorities and physicians; increased funding for basic research into the mechanisms of resistance; more adequate surveillance systems for tracking the spread of resistance; and a fast track for new antibacterial agents so that they become available for compassionate use in emergency situations.

In December of 1993, Tomasz moderated a New York Academy of Medicine conference addressing antibiotic resistance in New York City clinics and hospitals. The leading participants—who included Dr. Richard Roberts of New York Hospital and Dr. Barry Kreisworth of the Public Health Research Institute, as well as officials from the New York City Department of Health—felt such a sense of urgency that, one month later, in January, they formed the Bacterial Antibiotic Resistance Group (BARG). The aim of BARG, a grass-roots collaboration of the public and private sectors, is to accurately tally antibiotic-resistant bacteria in New York City; to improve infection control; to enlist the pharmaceutical industry and the Environmental Protection Agency; and to disseminate the techniques of molecular epidemiology to New York City hospitals.

In February, Tomasz flew to San Francisco where his presentation on "Disease-causing Bacteria Resistant to Antibiotics" was the most talked-about lecture at the annual meeting of the American Association for the Advancement of Science. Tomasz's warning about resistance became the number one story to emerge from the conference, reaching an estimated audience of 42 million people worldwide. Coverage was so widespread that Washington began to pay attention, and in March, Tomasz was contacted by the Office of Technology Assessment to help them prepare a report to Congress on the public health dimensions of bacterial antibiotic resistance.

Tomasz has been active internationally as well. Together with his friend, the South African scientist Keith Klugman, he organized in April of last year a conference in Prague focusing on the multidrug-resistant pneumococcus in Eastern Europe. Tomasz, who had marshaled a network of investigators to collect data on resistance in the former Eastern Bloc countries, found signs of increasing resistance in Hungary, Croatia, Bulgaria and Slovakia.

Above, Dr. Tomasz surrounded by some members of the microbiology laboratory who hail from 11 countries.



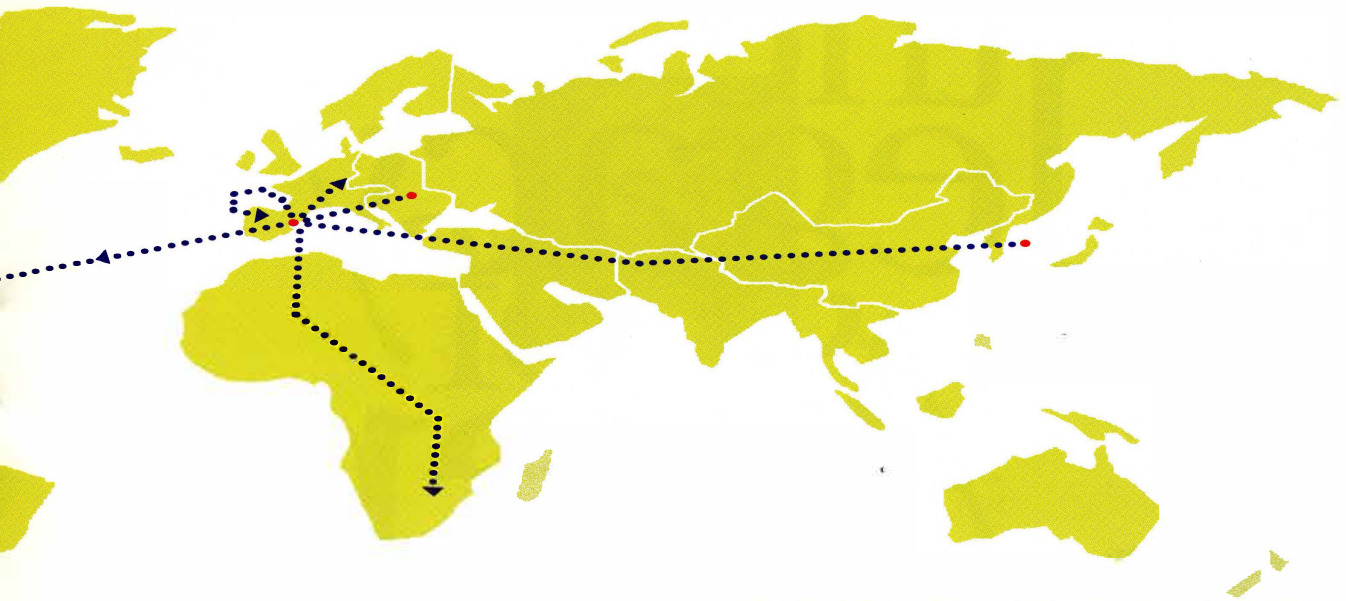
A few days later in May, while still in Prague, Tomasz was a plenary speaker at the International Society of Infectious Disease conference addressing the global threat posed by bacterial resistance. Both Prague meetings were standing-room-only events, generating much professional interest.

Instead of indiscriminate killing with drugs, which inspires germs to develop and share new resistance strategies, more selective approaches are called for.

In July, Tomasz, along with Nobel laureate, Rockefeller University professor and former president Joshua Lederberg, was asked to serve on a new task force on antibiotic resistance organized by the American Society of Microbiology. Lederberg was coauthor of the seminal 1992 National Academy of Sciences report, "Microbial Threats to Health in the United States," which first brought national and international attention to the larger issues of pathogenic microbes. Lederberg's laboratory was also one of twelve at Rockefeller that joined with Tomasz to start a new campus research initiative on microbial antibiotic resistance and disease.

New Hope: Back to the Future

The aim of the new Rockefeller research program that Tomasz has proposed is to develop knowledge and strategies for combating the five types of resistant bacteria that pose the greatest threat to public health: tuberculosis, *Staphylococcus aureus*, coagulase-nega-



Above, Worldwide spread of a multiply drug resistant clone (capsule type 23F) of pneumococcus, probably originating in Spain. Using molecular "fingerprints" to identify the offspring of drug-resistant pneumococcus, the Tomasz lab has helped track the disease-causing bacteria all over the globe.

tive staphylococci, pneumococci, and enterococci.

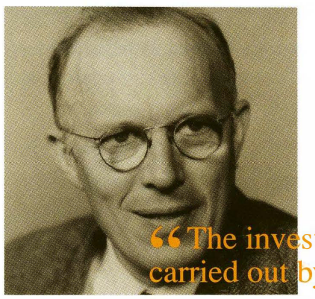
"Such a program should revitalize interactions among laboratories with complementary skills," says Tomasz. "It will also provide the structure for a modern and exciting interdisciplinary training program for graduate and postgraduate trainees. In addition, clinical research in antibiotic resistance will be expanded at The Rockefeller University Hospital." The plan also includes studies in the molecular epidemiology of resistance to be conducted in collaboration with several hospitals in the U.S. and abroad.

It is no coincidence if the mission behind these initiatives sounds familiar. As Rockefeller University President Torsten Wiesel told participants at the July 1993 workshop, it was the deadly epidemics of infectious diseases that prompted the foundation of The Rockefeller Institute for Medical Research in 1901 as the nation's first biomedical research center. "The founders of the Institute understood clearly that the ultimate control of these devastating microbial diseases required first that scientists turn their attention to the understanding of the nature and mechanism of infectious disease," said Wiesel. It will be the mission of modern microbiologists at Rockefeller and elsewhere, he emphasized, to readdress themselves to this public health issue now, with emphasis on antibiotic-resistant microbes that cause invasive diseases.

Tomasz believes that the best hope lies in rethinking our entire approach to treating infectious diseases. Instead of indiscriminate killing with drugs, which inspires germs to develop and share new resistance strategies, more selective approaches are called for. "For example, you could aim drugs at the specific disease-causing components of the bacteria, or at the dangerous response they provoke in the host," he suggests. "The result would be more selective, lower profile drugs that would threaten a bug's bad habits rather than its survival, making the drugs far less likely to become sitting ducks for resistance." Tomasz is convinced that the design of such future drugs will require that the pharmaceutical industry develop close alliances with microbiology laboratories studying mechanisms of bacterial physiology, gene transfer and disease.

In his own efforts to find new molecular strategies that can be mobilized against bacteria, Tomasz has reinstated studies in his lab on genetic transformation. So he, and other pneumococcal researchers at Rockefeller, have truly come full circle. Pneumonia, the leading killer at the beginning of the century, led to Avery's pioneering work on pneumococcus and the discovery that genes are made of DNA. Now DNA techniques are helping Tomasz and others study the pneumococcus with greater precision in an attempt to better understand antibiotic resistance and conquer pneumococcal disease. Says Tomasz, "That incredible little bug, the pneumococcus, still has many lessons to teach us." S

the legacy of Avery

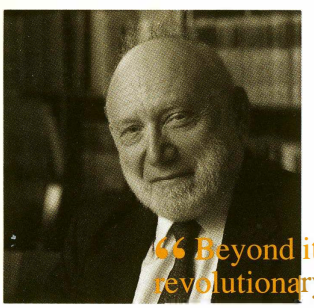


Rockefeller University Archives

"The investigations carried out by Avery and his school between 1913 and 1940 have provided the pattern, the master plan, used by our generation for the immunochemical study of infectious processes." 1976

René J. Dubos

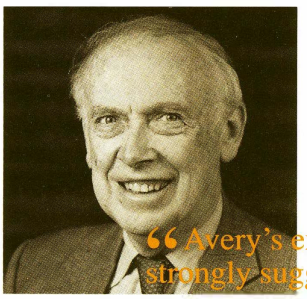
microbiologist, The Rockefeller University



"Beyond its details, the revolutionary contribution of Avery, MacLeod and McCarty was the refocusing on DNA by a generation of chemical biology. Certainly that was its precise impact on the initiation of my own scientific career." 1979

Joshua Lederberg

Nobel laureate, former president of The Rockefeller University, on the 35th anniversary of the Avery discovery

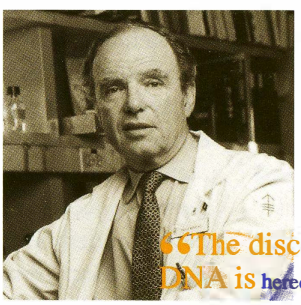


Cold Spring Harbor Laboratory Research Library Archives

"Avery's experiments strongly suggested that future experiments would show that all genes were composed of DNA. If true, this meant to Francis [Crick] that proteins would not be the Rosetta Stone for unravelling the true secret of life. Instead, DNA would have to provide the key to enable us to find out how the genes determined, among other characteristics, the color of our hair, our eyes, most likely our comparative intelligence, and maybe even our potential to amuse others." 1968

James D. Watson

Nobel laureate, in The Double Helix



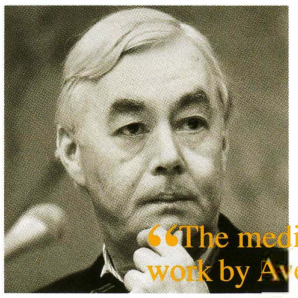
Adam Stoltman

"The discovery that DNA is hereditary material [is] perhaps the most important discovery in biology of the 20th century." 1985

Paul A. Marks

M.D., president, Memorial Sloan-Kettering Cancer Center

what they said

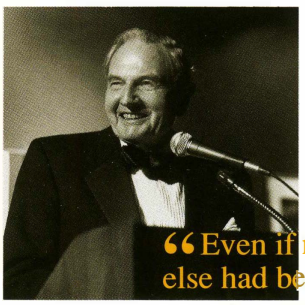


Courtesy of Daniel P. Moynihan

"The medical research work by Avery, McCarty and MacLeod conducted at Rockefeller University during World War II changed the course of the world, reduced suffering and contributed immeasurably to the quality of life as we know it." 1994

U.S. Senator Daniel P. Moynihan

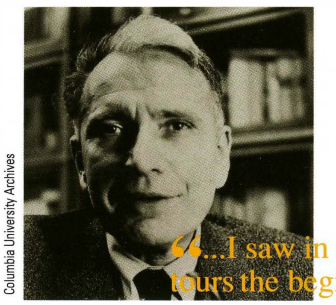
entered into The Congressional Record, February 2, 1994



"Even if nothing else had been done at this great university, this extraordinary discovery has, in my judgment, more than justified—all by itself—the great hope and aspiration of my grandfather and father when they established this institution in 1901. It has given to the world what they hoped for: the beginning of the understanding of the inner mysteries of life and disease." 1994

David Rockefeller

chairman of the Executive Committee of the Board of Trustees, The Rockefeller University

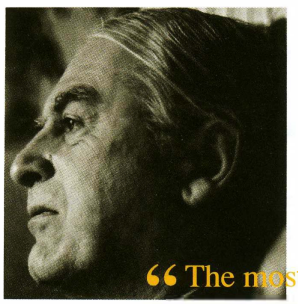


Columbia University Archives

"...I saw in dark contours the beginning of a grammar of biology....Avery gave us the first text of a new language, or rather he showed us where to look for it." 1971

Erwin Chargaff

professor emeritus, Columbia University

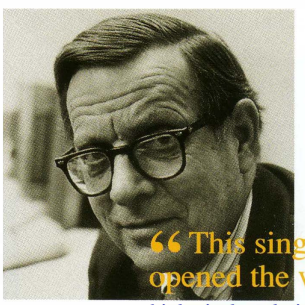


Caroline Garland

"The most interesting and portentous biological experiment of the 20th century...." 1985

Sir Peter Medawar

Nobel laureate, immunologist



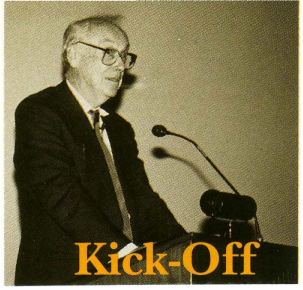
"This single discovery opened the way into the biological evolution which continues to transform our view of nature in its most intimate details...." 1985

Lewis Thomas

physician, scientist and essayist

a year long series of events

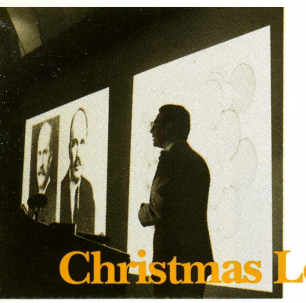
celebrating 5



Kick-Off

November 19, 1993

James D. Watson, Nobel laureate and director of the Cold Spring Harbor Laboratory, launches the celebration with a public lecture remembering the early days of DNA.



Christmas Lectures

December 29 & 30, 1993

Above, as part of a year-long focus on DNA research, molecular DNA researchers John Kuriyan and Stephen K. Burley discuss "da Vinci and Darwin and the Molecules of Life" at the annual Alfred E. Mirsky Christmas Lectures.

Round Table

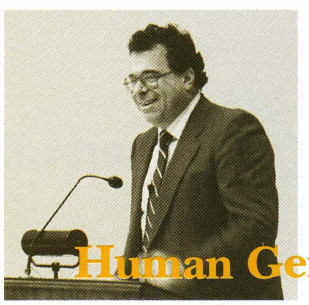
February 3, 1994

Nobel laureate Alfred Day Hershey, *left*, and Professor Emeritus Rollin Hotchkiss were among six pioneers active in the field of genetic research between the publication of the Avery paper and the discovery of the double-helical structure of DNA who gathered for a round-table discussion. The others were Visiting Professor Robert Olby, chair; Professor Joshua Lederberg; Professor Emeritus Maclyn McCarty; Erwin Chargaff; and Seymour Cohen.

Anniversary Day

February 1, 1994

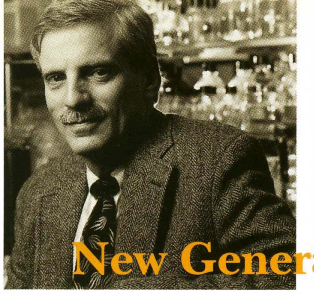
On the anniversary of the publication of the landmark paper, Professor Emeritus Maclyn McCarty, *center*, the sole surviving member of the Avery team, is honored in the RU Hospital by, *from left to right*, Deputy Mayor of New York City John Dyson, Chairman of the Board's Executive Committee David Rockefeller, President Torsten Wiesel, and Physician-in-Chief Jules Hirsch.



Human Genome

February 2, 1994

David Botstein, professor and chair of the genetics department at Stanford University, delivers a public lecture on the Human Genome Project.

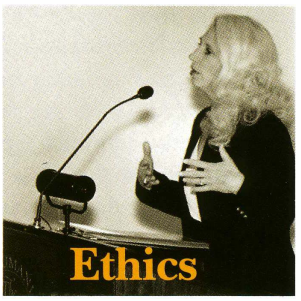


New Generation

May 6, 1994

Professor Jan Breslow, ***above***, chairs a scientific symposium featuring lectures by a new generation of DNA researchers at the university: Professor Stephen K. Burley, Associate Professor Frederick Cross, and Associate Professor Jeffrey Friedman.

0 years of DNA



Ethics

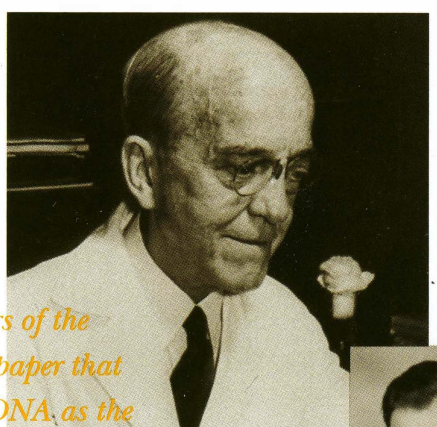
April 18, 1994

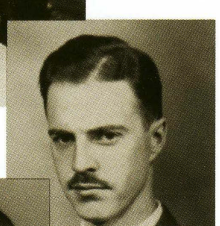
Nancy Wexler, professor at Columbia University and chair of the Human Genome Project's Committee on Ethical, Legal, and Social Issues, speaks on DNA technology and its consequences at a public lecture.

Immunology

February 4, 1994

Professor Emil Gotschlich, ***center***, chairs a scientific symposium that features Robert Austrian of the University of Pennsylvania School of Medicine, ***left***, and John Robbins of the National Institutes of Health. The symposium reviewed Avery's scientific accomplishments and subsequent developments in the fields of immunology and infectious disease.





The authors of the landmark paper that identified DNA as the hereditary material in genes: clockwise, *Oswald T. Avery, Colin MacLeod and Maclyn McCarty.*

Opening Pandora's Box: Nancy Wexler's Quest for the Huntington's Disease Gene

By Mika Ono Benedyk

The 1944 Avery paper did not just usher in a new era of molecular biology and genetics. The revolution in knowledge also created a host of new ethical dilemmas never encountered before. As part of its "50 Years of DNA" celebration, The Rockefeller University invited renowned Columbia professor and bioethicist Nancy Wexler to discuss some of the personal and ethical ramifications of our brave new genetic world.

Of all possible fates, having the genetic disorder that causes Huntington's disease is one that no person would choose. The symptoms, which usually set in at mid-life, are subtle at first: lack of coordination, small twitches, memory loss, depression, irritability. But as the disease progresses—over the course of 10 to 20 agonizing years—part of the brain inexorably degenerates. Those afflicted lose the ability to control their movement and their arms and legs flail about wildly. Near the end, and the end is inevitably death, patients are emaciated, incontinent, unable to speak, yet still able to grasp the tragedy of their demise.

Nancy Wexler, who spoke at The Rockefeller University in April as part of its 50th anniversary celebration of the discovery there that genes are made of DNA, has been at the forefront of bringing modern genetic technology to bear on this disease. Her work helped to find a genetic marker for the illness, and then to identify the genetic defect that causes Huntington's disease. While these discoveries have brought new hope for a cure, they have also brought agonizing new dilemmas for society.

A Personal Quest

When Wexler was 23, she learned that her mother, like three of her uncles and her grandfather, had Huntington's disease. In itself the news was devastating. But it also had implications for her sister and herself: it meant that they each had a 50 percent chance of developing the illness themselves later in life.

The days—and years—after Wexler's mother's diagnosis were trying. Wexler and her sister decided immediately never to have children.

> When Wexler was 23, she learned that her mother, like three of her uncles and her grandfather, had Huntington's disease.

Wexler battled depression. But everyone in the family also became passionately committed to the struggle against the disease.

Wexler, who had just graduated from Radcliffe, continued with her plans to pursue a Ph.D. in psychology, focusing on families with Huntington's. After graduating from the University of Michigan in 1974, she moved to New York to teach and open a private practice. It wasn't long before she was appointed executive director of Congress's Commission for the Control of Huntington's Disease and Its Consequences. After the commission's work was over, she became a health science administrator heavily involved in Huntington's issues at the National Institute of Neurological, Communicative Disorders and Stroke.

The Search for the Gene

Wexler had great faith that DNA could provide the clue to curing Huntington's disease. She felt that finding the gene would lead directly to a treatment. But for many years, the task seemed Herculean: Huntington's disease could be caused by a flaw in any one or several of the 3 billion base pairs in the human genome.

Then, in the early 1980s, fresh hope appeared. David Botstein (who also spoke at Rockefeller as part of the "50 Years of DNA" celebration) and his colleagues had developed a hypothesis that variability in the DNA close to a disease-causing gene could be used to demarcate the gene's approximate location. Disregarding the warnings of many reputable scientists that finding a marker could take over half a century, Wexler pushed ahead.

Mika Ono Benedyk, *a writer, works at the Rockefeller Group.*

Rob Marinissen

The search took her to the shores of Lake Maracaibo, in Venezuela. There, in several small, poor fishing villages, lay one of the largest communities in the world afflicted with Huntington's disease. Throwing themselves into the quest, Wexler and her colleagues built a pedigree of almost 13,000 people and collected blood samples which would come to number over 3,000. Jim Gusella at Massachusetts General Hospital, who was also studying a family with Huntington's from Iowa, extracted the DNA from the Venezuela samples and looked for a genetic marker that "traveled" with Huntington's disease in a family.

In an incredible stroke of luck, the Gusella lab together with P. Michael Conneally of Indiana University stumbled upon a mark er on the 12th try. Almost all those with Huntington's disease had one form of the marker while their healthy relatives had another. Wexler was jubilant. The location of the probe was quickly mapped to the top of the short arm of chromosome 4, and the results were published in *Nature* in November 1983, just three years after the effort began.

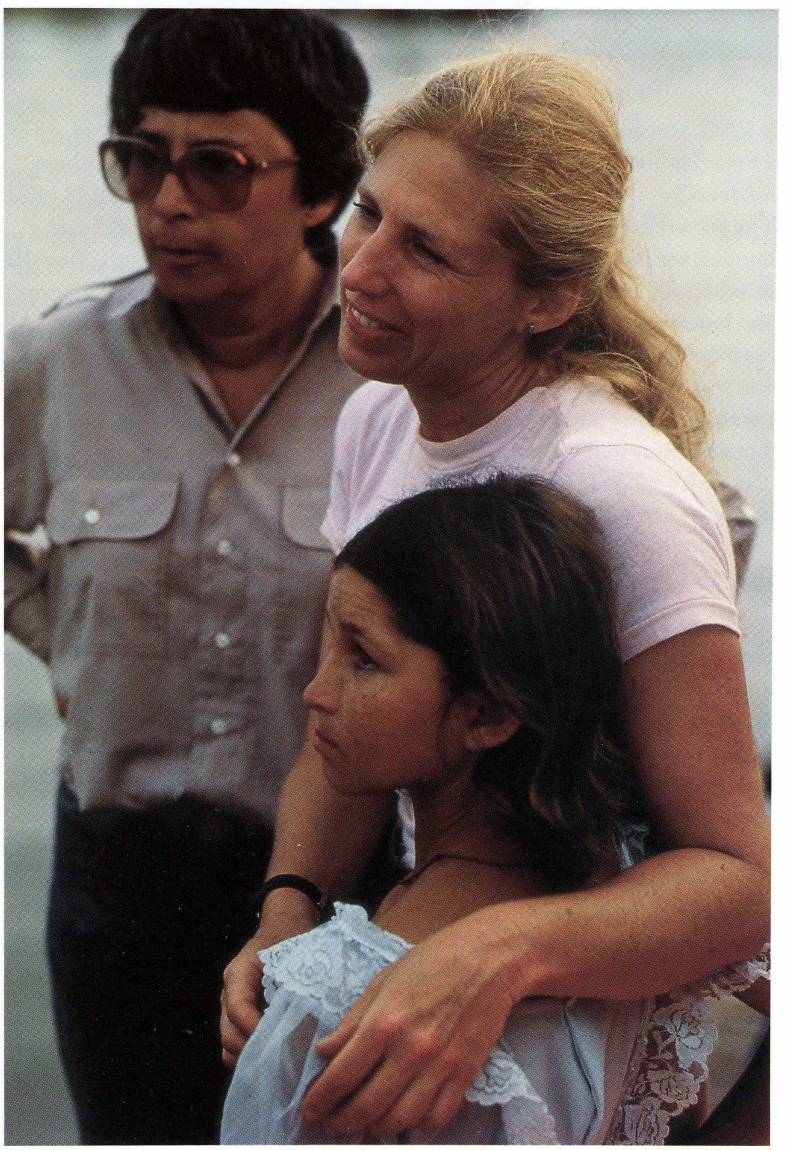
Eureka!

The next goal was to find the exact location of the gene, isolate it and learn its secret—what Wexler at first assumed would be a quick and easy task.

Wexler helped marshal the forces of six scientific groups around the world to collaborate instead of compete in their search for the gene, a highly unusual effort in the often cutthroat arena of science. The groups were led by: Francis Collins of the University of Michigan; Hans Lehrach of the Imperial Cancer Research Fund, London; Peter S. Harper of the University of Wales College of Medicine; David Housman of the Massachusetts Institute of Technology; Gusella; and John Wasmuth of the University of California, Irvine. One year stretched into two, then three... Almost 10 years passed without isolating the gene. Some scientists speculated that there was no Huntington's disease gene. Wexler likened the effort to "crawling up Mount Everest."

The Gusella group was focusing on one region of the chromosome as part of the collaborative effort. One night, Marcy MacDonald and Christine Ambrose of Gusella's lab discovered the end of a gene where there were 48 repeats of one sequence of nucleotide bases in a DNA sample from a Huntington's disease sufferer: cytosine, adenine, guanine (CAG). They checked against a sample from someone without Huntington's: There were only 18 repeats of this sequence. Could it be so simple? Soon, the researchers were calling Wexler with the good news: They had found the long-sought gene.

Wexler is still filled with wonder at the finding. "It strikes me as staggering the enormously subtle difference between life and death," she said. "If you have 11 to 34 repeats



Nick Kelsh

Above, Nancy Wexler sits behind a sculpture of the DNA double helix. *Right,* Wexler's quest for the gene that causes Huntington's disease took her to Lake Maracaibo in Venezuela, one of the largest communities in the world afflicted with the disease.

Peter Ginter

Above, Wexler hugs a Venezuelan child who carries the Huntington's gene.

of this CAG, you are going to live. If you have 3 more, from 37 to 100, you are dead."

The researchers also found that the youngest victims had the most repeats (although the oldest did not have the fewest), and that the number of repeats tended to expand slightly as the gene was passed from generation to generation. The Huntington's Collaborative Research Group published their conclusions last year in the journal *Cell.*

The Dilemmas of Testing

Although from a scientific perspective the advances in Huntington's disease have been astounding, from a medical point of view little has changed. There is no cure. There is no treatment. There is now only a genetic test that can tell individuals, before they develop symptoms, whether they will come down with the disease at some undetermined time later in life. The test itself poses new dilemmas for those at risk for the disease.

"When [the test] first became available, my sister, father and I had no question about taking it," said Wexler in an interview with *Columbia*, the magazine of Columbia University where Wexler is now a professor. "My sister and I thought, 'Isn't this fantastic!' We could have children! My father could stop saving money for nursing homes and retire. When we thought more about it, however, my father was the first to say, 'Wait a minute! I don't want to know if either of you has a bad outcome. One bad outcome and we're all three dead.'"

Soon we will all be facing the question of whether we want to know what is ahead in our own and our children's future.

As Wexler acknowledges, there are many compelling reasons for those at risk to take the test. The absence of the Huntington's gene would be a tremendous relief. Even if the results show the presence of the genetic defect, one's family, career and finances could more easily be planned.

On the other hand, the reasons for not taking the test can be just as compelling. To learn that you have the Huntington's gene and will die an early and prolonged death can be devastating for you and your family: Some who have tested positive have attempted suicide. And even a positive result leaves uncertainty: You do not know when the disease will begin its insidious advance. In fact, some individuals who know they have the genetic mutation begin to think of themselves as sick before they come down with symptoms, adopting a "sick identity" in the face of the agonizing ambiguity about when the disease will strike.

Even individuals who test negative are not always as euphoric as

they had thought they would be. They may feel guilty that siblings and parents have the Huntington's gene when they don't. Or they may have built their identities around being at risk—abandoning commitments, forgoing children, living life in the fast lane—and be unprepared to learn that they are ordinary, vulnerable to other diseases, and responsible for their lives and futures.

The prenatal test for Huntington's poses a similar dilemma. On the one hand, you can have a fetus tested for the Huntington's gene so that you won't bring children into the world who will later develop the disease. On the other, there is no medical justification to take the test unless you are willing to abort a fetus with the genetic flaw.

Underlining how personal and difficult these dilemmas are, Wexler will not say whether she has taken the test for the Huntington's gene, only that these decisions are complex and can change given different circumstances and period in the life cycle.

The genetic underpinnings of more and more diseases, such as heart disease, diabetes, colon cancer and breast cancer, are being revealed. For some of these diseases, early diagnosis can prove lifesaving. Soon we will all be facing the question of whether we want to know what is ahead in our own and our children's future, and whether we can do anything to change it.

A New Framework is Needed

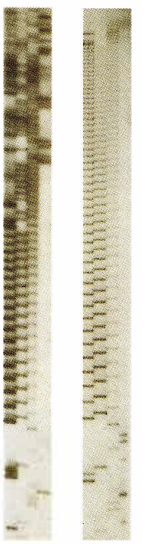
A cure for Huntington's, and other genetic diseases, would eliminate the dilemmas of testing in a single stroke. But such developments are a long way off. Researchers at The Rockefeller University and other institutions are exploring the possibility of using gene therapy—where healthy genes are inserted into a sick person's body—to treat certain genetic diseases such as cystic fibrosis. But such experiments are still in very preliminary stages, and treating a neurological disease such as Huntington's would pose many additional challenges as the brain is a very delicate and complicated target.

In the meantime, Wexler believes that we need to face the widening gap between our biological understanding and society's ability to encompass these new developments. As chair of the joint National Institutes of Health/Department of Energy Ethical, Legal and Social Issues Working Group, Wexler has struggled with responding to some of the new issues posed by genetic technology.

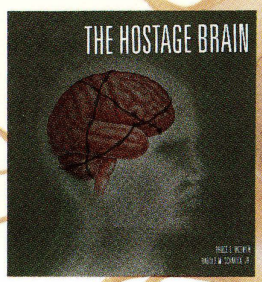
These challenges include ensuring that those being tested understand the risks of learning their genetic status and providing enough counseling to help them deal with the results. Other challenges are protecting the privacy of genetic information and restricting its use so that it cannot be used to deny employment, stigmatize individuals for having "undesirable" genes or prevent access to health care.

Wexler is especially worried about the potential for abuse by insurance companies. As it is, she points out, she herself would normally be totally uninsurable—not because she is sick, but because of her genetic risk. As genetic technology advances, insurance companies will have more and more opportunities to deny or limit coverage. "We have to be very explicit and very unified in fighting this kind of pernicious activity that distorts the way health care is delivered in this nation," she said.

Is Wexler sorry that she helped open Pandora's box, playing a part in ushering in this uncharted new age of genetic information? While she admits that for a time we may have the worst of all possible worlds—limited or no treatments, unrealistic expectations, insurance repercussions—she is still optimistic about the future. One day, she hopes, genetic technology will lead to a better world, one in which people will no longer have to suffer the worst of the fates written in their genes. S



Above, DNA sequence analysis of the $(CAG)_n$ repeat. In a person without Huntington's disease, there are only 18 repeats (*left*); the DNA sequence of a person with the disease can have up to 100 repeats (*right*).
Huntington's Disease Collaborative Research Group



THE HOSTAGE BRAIN

by Bruce McEwen and Harold M. Schmeck, Jr.

New Book from RU Press Explores Multiple Influences Shaping Human Brain

Background, The brain contains neurons, which generate and transmit electrical signals to other neurons, and glial cells, which nurture and protect the neurons. Blood vessels bring oxygen and glucose to neurons and glial cells, and the blood also carries hormones to the brain.

Illustration by Lydia Kibiuk

"There is nothing in the known universe to compare with the human brain." So reads the opening of the new book, *The Hostage Brain*, written by Rockefeller Professor Bruce McEwen and veteran science journalist Harold M. Schmeck, Jr. In the 300 or so pages that follow, the authors proceed to show just how wondrous the human brain really is.

The new book, published by The Rockefeller University Press, describes multiple influences on the brain such as the surprising interactions that occur between the brain and the immune system, with hormones from the immune system affecting the brain, and the brain innervating cells of the immune system and bombarding them with neurotransmitters and hormones. The book also describes the devastating impact of neurological diseases such as Alzheimer's and schizophrenia. It chronicles the impact of our inner biological clocks on memory, alertness and mood. It tracks the effect of aging on the brain. And it explores the impact of a wide range of experiences such as learning, stress and interpersonal relationships on the body's most complicated organ.

Many of McEwen's insights into the hostage brain come from work in his own laboratory, which focuses on the effect of sex and stress hormones on the brain. He first caught the bug for communicating these insights when he gave the Alfred E. Mirsky Christmas Lectures for High School Students in 1973. Three years ago, McEwen teamed up with Schmeck, a veteran science reporter who had recently retired from *The New York Times*. Schmeck's distinguished career covering scientific research provided him with a treasure trove of experience and anecdotes to make the science come alive. Together, the two authors worked with scientific illustrator Lydia Kibiuk to produce a book that aims to "reach out to every level of scientific understanding, and to allow people who are not scientists to learn about the brain." McEwen hopes that

Above, Dr. Bruce McEwen, (*right),* and Harold M. Schmeck, Jr., at the book launching party.

the book will be read by interested lay people, and also used in high schools, adult evening schools and certain medical school settings.

Each audience may take from the book different levels of understanding, but McEwen hopes that every reader will hear the book's fundamental message. He says, "If you're a hostage, then there has to be a ransom, or a way of breaking the bonds. And that really has to do with using our intelligence to understand how the brain works. Many of our most vexing problems—violence on our streets, stress in our lives, anxiety, mental illness—are problems of behavior and brain function that can be addressed more effectively by understanding how the brain operates and how it is capable of changing.

"The challenge to all of us is to use our brains to understand our brains and our behavior, because, in the end, our brains are mainly hostage to one thing: ignorance."

Hardcover ($39.95) and softcover ($19.95) copies of The Hostage Brain *are available from The Rockefeller University Press Order Service, 212.327.8572 or fax 212.327.7944.*

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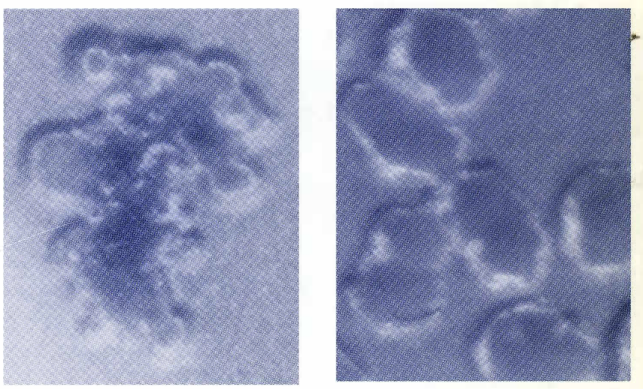

Blue colonies of a pneumococcal strain with a genetic defect in an exported protein, *left*, compared to the white parental strain (magnification: 20). Capitalizing on the 1944 discovery of Avery, MacLeod and McCarty, Rockefeller University investigator Robert Masure designed a genetic strategy in 1994 to systematically map the surface of pneumococcus.