

SPRING 2005

Life sciences at Whitehead Institute for Biomedical Research

paradigm

The double life of

CHRISTOPHER HUG, M.D., Ph.D.

PLUS

RNAi for clinics?

**How obesity
feeds diabetes**



Window on Whitehead

Stemming the tide

GOVERNOR MITT ROMNEY was blunt: In embryonic stem cell research, “there is an ethical boundary that should not be crossed.”

Speaking to the *New York Times* in February, Romney declared that research institutions in Massachusetts had crossed that line, and that he would introduce legislation that would turn some of our planned projects into criminal acts.

Romney’s declaration reignited this state’s long-simmering brushfire about human embryonic stem cell research, sparking a brief but intense firestorm of front-page news and battling advertisements.

The governor claimed to represent a middle ground in the controversy. He approved studies with human embryos that otherwise would be discarded by in vitro fertilization clinics. He drew the line at somatic cell nuclear transfer, also known as therapeutic cloning.

In this process, the nucleus is removed from an egg and replaced with the nucleus of an adult cell, kicking off a growth cycle that produces a blastocyst, a cluster of developing cells. When the blastocyst is suitably cultivated in a Petri dish, stem cells emerge and start dividing. Scientists then can struggle to differentiate the stem cells into the kinds of cells they want to study.

But this research raises serious issues on which people may reasonably disagree. Some see the blastocyst as a human embryo, and are adamant that we thus should not create a human life in order to destroy it during research, however appealing the research goals might be. “Science does not have to kill in order to cure,” as the state’s Roman Catholic bishops put it. Many also worry that somatic cell nuclear transfer steps onto a slippery slope toward reproductive cloning, since that process also starts with the creation of a blastocyst.

The Bay State’s public debate did not always hit the highest level, especially when opponents of the research ran ads that misrepresented its history. The *Boston Globe* commissioned a muddled survey of Massachusetts voters and gave short shrift to other surveys with conflicting results. (As always, survey results depend strongly on how you ask the question. In this case, the approval rate seems to soar when the biology is understood.)

Whitehead joined a coalition of research institutions, hospitals, patient organizations and the Greater Boston Chamber of Commerce to engage the State House. We were pleased that in March, bills allowing nuclear transfer passed both houses by large majorities. Such legislation now seems likely to become law over Romney’s veto.

Does this story have a happy ending? No, it’s not an ending. This debate will move back to the federal level.

But it does have a moral: The price of scientific freedom is unending vigilance by the scientific community. As our understanding of biology grows, so does the political resistance to the choices it brings. If you believe that stem cell research is proceeding thoughtfully and appropriately, you can’t be shy about voicing that belief.

Eric Bender
Acting director, Communications and Public Affairs



on the cover

Who works harder than an MD? An MD who’s also doing serious lab research. Christopher Hug is one of a long line of Whitehead physician-scientists. Photo by Kathleen Doohar.

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cover story

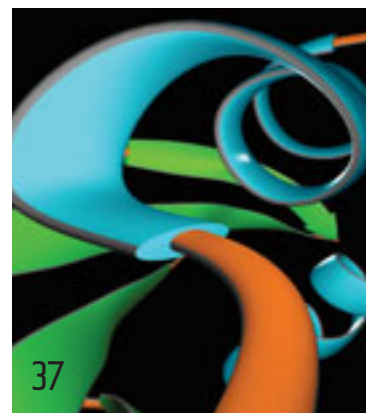
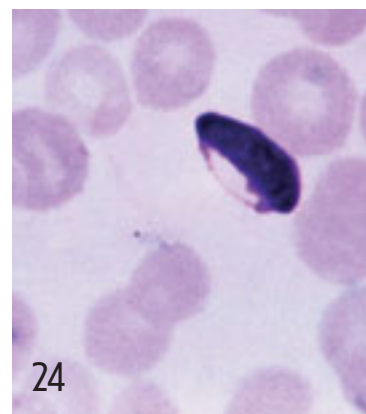
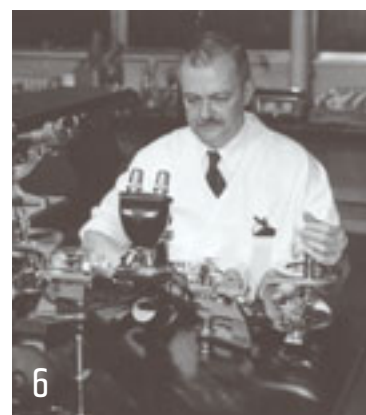
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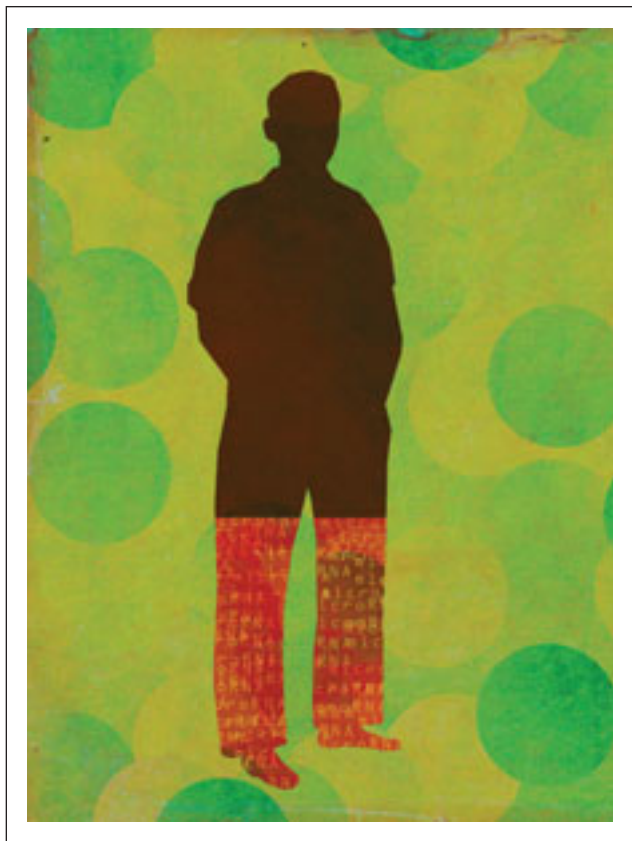
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BY DAVID CAMERON



RNA grabs the driver seat

MicroRNAs found to regulate nearly one-third of human genome

FOR MANY YEARS, DNA and proteins have been viewed as the real movers and shakers in genomic studies, with RNA seen as little more than a messenger that shuttles information between the two. But researchers from Whitehead and the Massachusetts Institute of Technology have discovered that small RNA molecules called microRNAs regulate thousands of human genes—more than a third of the genome’s protein-coding regions.

“It’s exciting to see how many genes are regulated by microRNAs. We now know that this type of gene control is much more widespread than previously appreciated,” says Whitehead Member and MIT professor of biology David Bartel.

MicroRNAs interrupt a gene’s ability to make protein. These tiny, single-stranded pieces of RNA are newcomers to biological research. It wasn’t until 2000 that researchers even knew that microRNAs existed in humans. But recently in the journal *Cell*, Benjamin Lewis, a graduate student working jointly with Whitehead’s Bartel and MIT associate

“As more genome data becomes available and the technology becomes more sophisticated, I think we’ll find even more genes targeted by microRNAs.” —Benjamin Lewis

professor of biology Christopher Burge, provided the first evidence that microRNAs influence a large percentage of life’s functions.

The team developed a computational method to define the relationship between microRNAs and their target genes. In December 2003, the same group identified 400 genes in the human genome targeted by microRNAs. (Prior to this study, there were no known microRNA targets in any vertebrate.)

In their latest paper, the team has compared human genome data with that of the dog, chicken, mouse, and rat. For each of the microRNAs and protein-coding genes that are common to these five species, the team looked for correspondence between the microRNAs and the protein-coding genes.

They discovered that regulation of a third of these genes has been preserved since the last common ancestor of mammals and chickens, which lived 310 million years ago.

“This study is an excellent example of the power of comparative genomics to illuminate how human genes are regulated,” says Burge.

“As more genome data becomes available and the technology becomes more sophisticated, I think we’ll find even more genes targeted by microRNAs,” predicts Lewis.

In addition, the team discovered some hints about how microRNAs find their targets.

To produce a protein, the cell first makes a template for that protein by constructing a molecule called messenger RNA. MicroRNAs associate themselves with particular messenger RNAs, thereby reducing the amount of protein that’s ultimately produced.

In this study, the researchers determined which portion of the microRNA is most important for this process, and identified additional determinants in the messenger RNA that are likely to contribute to recognition by microRNAs.

These findings contribute to the recent interest in potential therapeutic uses of RNA.

For example, using a technique known as RNA interference, or RNAi, researchers are shutting off genes by delivering into cells artificial microRNA-like molecules called short interfering RNAs (siRNAs). RNAi has transformed the way that many labs are investigating gene functions, and siRNAs are being developed for clinical applications (see “Knockout punch” on page 10).

Learning more about how microRNAs operate in human cells should help scientists to understand how best to exploit siRNAs for treating disease.

Prions act as stepping stones in evolution

In yeast, misfolded proteins can express different genetic traits

WHEN A PROTEIN MISFOLDS, the results can be disastrous. An incorrect change in the molecule's shape can lead to diseases including Alzheimer and Huntington. But scientists have discovered that misfolded proteins can have a positive side in yeast, helping cells navigate the dicey current of natural selection by expressing a variety of hidden genetic traits.

What's more, at the center of this process is a prion, a protein that changes shape in a self-perpetuating way—much like the prion in mammals that is responsible for certain neurological conditions such as Mad Cow disease.

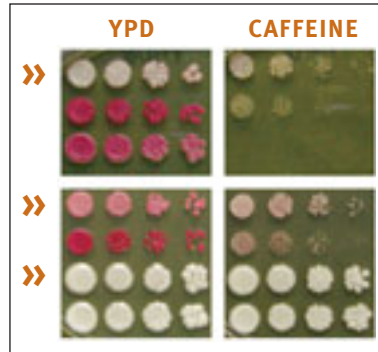
"This is the first time we've seen a prion affect a cell in a beneficial way that can determine the evolution of an organism," says Heather True, lead author of a paper on protein folding in

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yeast that appeared in the journal *Nature*.

Previously, True and Whitehead Member Susan Lindquist reported that a particular yeast protein called Sup35 somehow altered the metabolic properties—or phenotype—of the cell when it "misfolded" into a prion state. Sup35 helps guide the process by which cells manufacture protein molecules. However, when Sup35 misfolds into its prion state, it forms amyloid fibers similar to those found in Alzheimer patients and causes the cell's protein-producing machinery to go drastically awry.

More often than not, this is deleterious to the cell. In about 20% of the cases tested, however, the Whitehead team discovered that these new phenotypes afford the yeast cell a survival advantage.



Yeast cells with a prion (rows marked with ») and without a prion respond to different environments. YPD (which stands for Yeast/Peptone/Dextrose) is a nutrition-rich environment for yeast, while caffeine is a more hostile environment. The top two images show how typical yeast cells respond to these environments over time. The cells with the prion do badly when exposed to caffeine. The two bottom images show strains that have evolved, which fare better under harsh circumstances.

"But we still didn't know the molecular mechanisms behind this," says True. A former postdoctoral researcher in the Lindquist lab, she is now an assistant professor at Washington University in St. Louis.

"How exactly did the prion change the appearance of the cell?" she asked.

The answer revealed a twist in the traditional understanding of how traits are inherited.

In order for Sup35 to ensure that the cell properly reads the protein recipes contained in genes, it focuses on what are called stop codons—sections of DNA that indicate exactly where in the gene a particular protein recipe ends. Sup35 ensures that the cell only translates material prior to these designated codons.

But when it misfolds into a prion conformation, Sup35 gets sloppy, and the cell reads beyond the stop codons, translating genetic information that previously had been dormant. As a result, the cell's phenotype changes.

Here's where evolution comes

in. On those rare occasions when, due to a particular environment, the altered properties of the cell provide it with a survival advantage, the cell passes those properties on to its progeny. But when

the daughter cells are mated and genetic reassortment takes place, they can subsequently pass along these same traits without the prion—that is, the traits become fixed in the cell's lineage and no longer depend on the prion state.

"We don't know yet exactly how the daughter cells do this," says Lindquist, who also is a professor of biology at MIT, "but they do it quickly, often after a single mating."

The prion thus appears to function as an evolutionary stepping stone, affording the cells a chance to survive in a new environment where they need a different phenotype until they can acquire the genetic changes that produce the same effect.

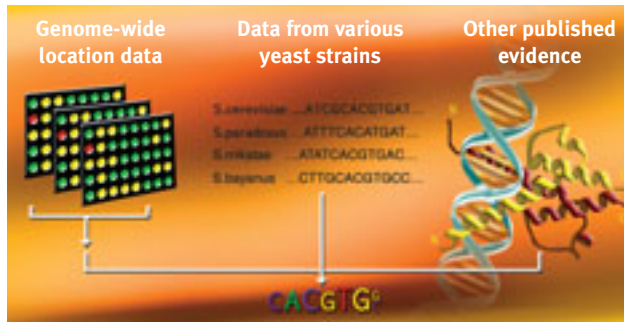
These new traits are genetically complex. When Sup35 misfolds into a prion form, it affects a number of genes in one fell swoop. "This prion," explains Lindquist, "has a capacity to hide and release genetic information throughout the entire genome that can contribute to new traits in a complex way."

Gearing up for the human genome

Researchers identify all the gene regulators for yeast

SCIENTISTS HAVE churned out genome sequences for everything from fungi to dogs to chimps. However, because a genome sequence is little more than a static list of chemicals—like, say, a parts list for a 777 airplane—scientists are increasingly turning their attention to figuring out how living organisms put their genes to work. Using yeast as a testing ground, White-

head scientists have for the first time revealed all the “controlling elements” of an entire genome—findings that may soon contribute to a new way of understanding human health and disease.



Combining microarray data from four species with previously published evidence, Whitehead scientists have mapped the yeast genome’s controlling elements.

head scientists have for the first time revealed all the “controlling elements” of an entire genome—findings that may soon contribute to a new way of understanding human health and disease.

“This is really the next stage in human genome research,” says Whitehead Member Richard Young, who headed the project together with Whitehead Fellow Ernest Fraenkel and MIT computer scientist David Gifford.

Key to understanding how the genome is controlled are gene regulators, also known as transcrip-

tion factors. These small molecules intermittently land on a region of DNA, close to a particular gene, and then switch that gene on. They can also influence the amount of protein that the gene will produce. Many diseases, such as diabetes and cancer, are associated with mutated gene regulators.

But very few of these regulators have been identified in any organism. Locating their landing sites is essential to identifying their function, and gene regulators are hard to find. They typically just land on a small stretch of DNA, do their job, and then take off again. And owing to the vastness of the genome, locating just one gene regulator with conventional lab tools can take many years. The Whitehead/MIT team, reporting in the journal *Nature*, described a method for scanning an entire genome and quickly identifying the precise landing sites for these regulators.

“We’ve located all 203 regulators in yeast,” says Young, who is also a professor of biology at MIT. Using tools developed in Fraenkel’s lab, the researchers also nailed down the exact landing points. As a result, scientists now can begin to understand how genes and their regulators “talk” to each other. Knowing these communication patterns ultimately will have a profound influence on our understanding of everything from infectious disease to cloning, Fraenkel predicts.

The next challenge is to scale the platform so it can tackle human cells, something that the researchers are gearing up to do. Even though the yeast genome’s 203 regulators are a far cry from the roughly 2,000 in human cells, Young explains, “now we have the technology and the concepts to get started on decoding the human genome.”

Sperm cells “spring” into action

Storage mechanism may point to applications in nanotechnology

SCIENTISTS HAVE identified a surprising mechanical means by which cells store and release energy, a tightly wound jack-in-the-box mechanism rather than the chemical storehouse cells are known to use. This process, observed in the sperm cells of horseshoe crabs, was imaged at nearly atomic scale by researchers from Whitehead and Baylor College of Medicine.

“This is nature’s prototype for how we can store energy and use it

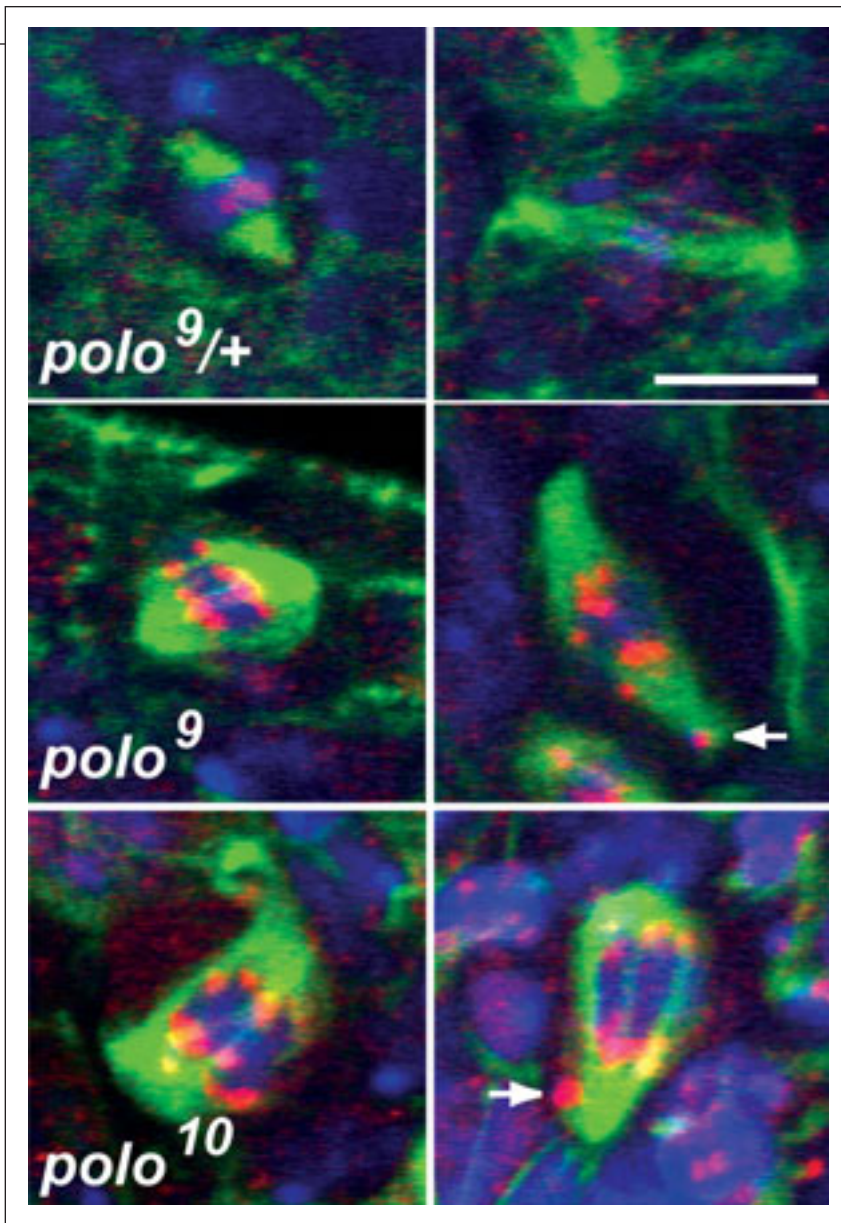
to do work,” says Paul Matsudaira, Whitehead Member and co-author of the study. “This finding tells us ways that we can think of powering nanomachines that we really hadn’t thought about before,” he adds.

Sperm cells need to work hard to penetrate an egg cell. The horseshoe crab takes a theatrical approach, using a molecular harpoon to spear through the egg’s membrane.

This harpoon comprises two proteins, actin and scruin, intertwined

in a bundle of filaments. Reporting last September in the journal *Ú*, the researchers describe the slightly irregular helical structure of this bundle, which “unlatches” to permit the sudden release of stored energy.

“Normally actin works by a chemical means, but this process doesn’t require chemistry,” says Michael Schmid, associate professor of biochemistry at Baylor College of Medicine, and lead author of the study. “It just requires mechanics.”



In the top two frames, the glue-like protein MEI-S332 (shown in red) leaves the chromosomes (shown in green) once they separate. In the bottom four panels, a mutant form of MEI-S332 still sticks, causing the chromosomes to separate abnormally.

plete maternal and complete paternal set of chromosomes.

To do this, the cell first enters into an intermediate stage where its number of chromosomes doubles, resulting in a single cell that for a brief time contains all the genetic material for two cells. During that time, the chromosomes are paired and attached to each other—mother to mother, father to father—by proteins that act as a sort of glue.

When the time comes for cell division to complete, the protein dissolves and releases the chromosomes from each other, allowing them to separate into the daughter cells. This protein-based “glue” is regulated by the MEI-S332 protein discovered in the Orr-Weaver lab and found in both fruit flies and mammals.

“The failure of this glue-like protein to function properly will result in cells with either too many or too few chromosomes,” says Astrid Clarke, a postdoctoral researcher in the Orr-Weaver lab and lead author on the paper. “Hence birth defects or cancer.”

Through studying how this protein functions in the fruit fly, Clarke identified the precise chemical reaction by which the protein binds and then releases the chromosomes during this intermediate stage. The key finding was that the protein releases the chromosomes from each other by adding a phosphate to the binding point.

These findings are particularly significant given that researchers have found that levels of MEI-S332 are higher than normal in 90% of all breast cancers. According to Clarke, this might mean that when there’s too much of the protein, the chromosomes don’t separate properly, or it might mean that the MEI-S332 gene is mutated on the chromosomes. Either way, “there definitely is a direct link between our protein and cancer,” says Clarke.

When cells divide

Studies of how chromosomes bind and release may shed light on cancer and birth defects

CELLS ARE DIVIDING all the time, and that’s a good thing. If they didn’t, our tissue and organs couldn’t replenish themselves, and pretty soon we’d be done for. But when cell division goes wrong, it can have disastrous results, such as cancer and birth defects.

Scientists in the lab of Whitehead Member and MIT professor of biology Terry Orr-Weaver have uncovered one of the primary mechanisms governing cell division, publishing the results earlier this year in the journal *Developmental Cell*.

“This paper advances our understanding of how the accurate partitioning of chromosomes is ensured during cell division by defining the mechanisms controlling a key protein in the process, MEI-S332,” says Orr-Weaver.

Except for egg and sperm cells, all of the chromosomes in our cells come in pairs. Half of each pair comes from our mother, and the other half from our father. When a cell divides to give birth to two daughter cells, it must ensure that both new cells also contain a com-

OPPOSITE: By 1919, Clarence Cook Little had developed stable lines of lab mice that aided early cancer studies.



The mighty mouse

HERE'S HOW OUR FAVORITE TEST MAMMAL
ROSE FROM HUMBLE BEGINNINGS TO SUPERMODEL
BY STEVE MIRSKY

In 2002, the mouse joined humans as the only mammals to have their genome sequences published. To celebrate, millions of mice went about their usual business in laboratories around the world. Countless more continued their tireless work outsmarting humans by living in our homes, eating our food and avoiding our traps.

The same issue of *Nature* that included the mouse genome featured a timeline of great moments in mouse history. It noted that the genus *Mus*, which includes most lab mice, was established some six million years ago. The timeline then helpfully explained that the name *Mus* “comes much later.” Everybody since T. S. Eliot

knows that only the cat—the mouse’s sworn cartoon enemy—actually names itself, so *Nature*’s explanation of the time gap was probably unnecessary. Anyway, *Mus* comes from the old Sanskrit word (there are few new Sanskrit words) “mush” or “musha,” which means thief.

Indeed, mice have probably raided

human provisions since humans had provisions. But with the advent of agriculture, *Mus* really hitched its tiny wagon to humanity—diving into storehouses of grain rather than foraging for whatever grasses were available. The ancient Roman naturalist Pliny reputedly supplied what became the species name, *musculus*—little mouse, or little thief, if one goes back to its true etymological mussy roots.

After hitching its wagon, *Mus musculus* hitched rides, following humans all over the planet. Mice have even been found living happily in housing in Antarctica.

Being cute and furry, mice also

CHRIS COLLINS/CORBIS

became tolerated in some circles. During the 17th century, Japanese aficionados collected mice with odd colorations or interesting behaviors. (What was later named the “waltzer” mouse seemed to dance, which was later found to be due to an inner-ear problem—one man’s waltz is another mouse’s struggle to keep upright.)

THE SCHOOL OF MICE

A hundred years ago, mouse fanciers also were active in the U.S. Which brings the story to Granby, Massachusetts, where a retired schoolteacher named Abbie Lathrop fouled up.

According to Karen Rader’s *Making Mice*, a history of how the humble house mouse became a laboratory star, Lathrop tried her hand at raising poultry. But in 1900 she chickened out and turned to a pair of mice. And flex her *musculus* she did, with a supply of some 10,000 in 1913. These mice were already part of the way to being ideal laboratory specimens—over hundreds of years people had selected those mice that didn’t mind being literally manhandled.

Harvard University biologist William Castle was buying mice from Lathrop as early as 1902, for early genetics research. And Castle soon had an ambitious, energetic student named Clarence Cook Little.

Little was smart enough to let his mice lead him around, just as Thomas Hunt Morgan had been clever enough to allow his fruit flies to alter his experimental objectives. Morgan originally tried to use flies to look at evolution, but his rapidly growing collection of mutant flies led him towards genetics instead. Castle and Little were hoping to use mice as the mammalian equivalents of flies to extend genetics. But the mice ultimately showed themselves to be even better if used for medical research.

Little spent years further inbreeding Lathrop’s already inbred mice, creating strains whose representatives were virtually clones of one another. By 1919 he had stable lines that were particularly prone to developing tumors. (Lathrop also found cancer-prone strains; she co-authored 10 papers on cancer heredity with Leo Loeb of the

University of Pennsylvania between 1913 and 1919.)

The hope was that study of these lines might lead to insights about human cancer development and treatment. It requires an effort today to imagine a time when the two disciplines had little to do with each other, but Little, along with researcher Ernest Tyzzer, “made some of the earliest conceptual connections between biological and medical research,” notes Rader, a science historian at Sarah Lawrence College.

ACTION AT JACKSON

In 1922, only 12 years after graduating from Harvard, Little became president of the University of Maine. The state’s oceanfront made it a favorite summer hangout for some of Detroit’s big automobile honchos, such as Henry Ford and one Roscoe Jackson, who owned the Hudson Motor Car Company. Little’s association with these Midwestern movers and shakers led him to the presidency of the University of Michigan just three years later. He took his mice with him to Maine and then Michigan, but the demands of administration obviously slowed his research and, at least in Michigan, rapidly began to lose their appeal.

During this period, he also envisioned a research colony in Maine that would be like a combination of the



Scientists now can choose from no fewer than 114 varieties of Jackson Lab mice—for diabetes research alone.

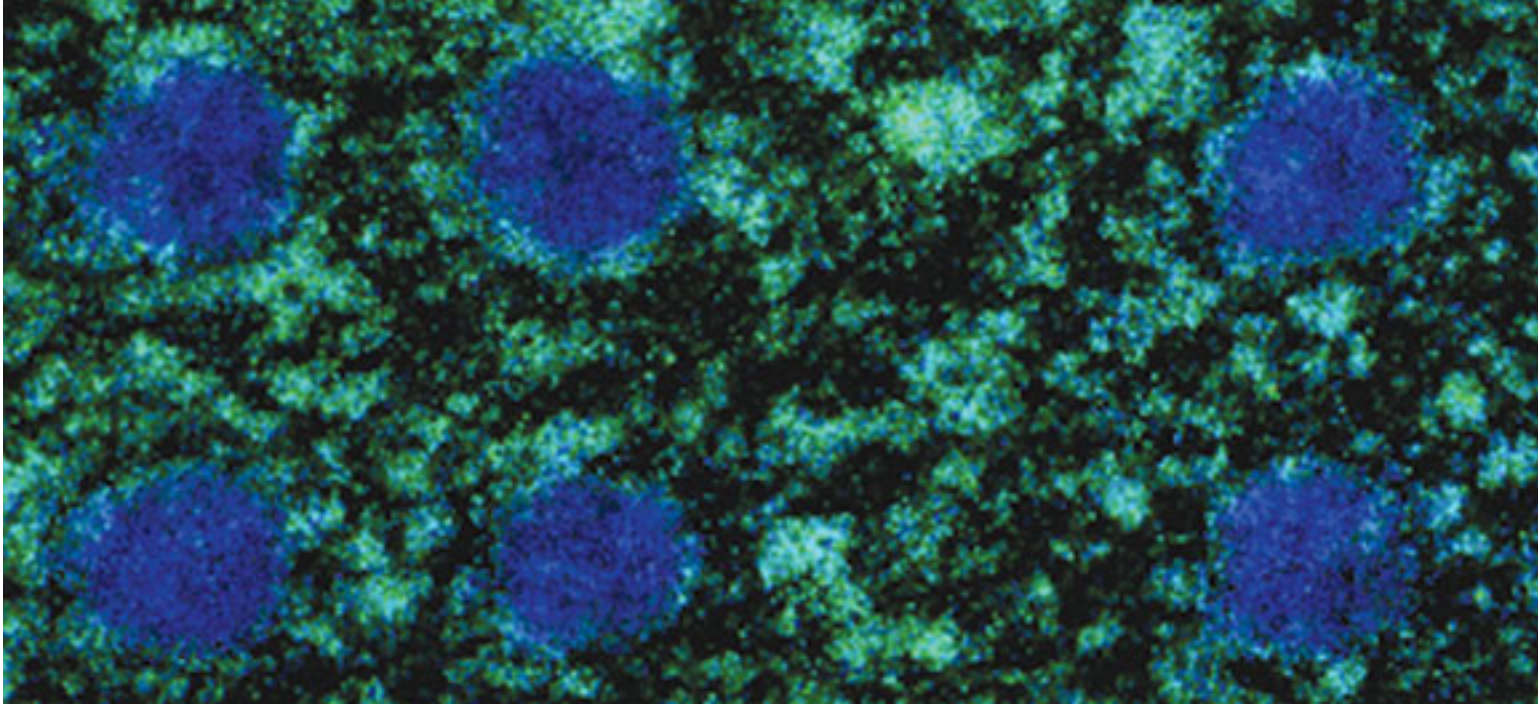
Marine Biological Laboratory on Cape Cod and New York City’s Rockefeller Institute for Medical Research (now Rockefeller University). He began pitching it to his wealthy car-maker comrades. It was Roscoe Jackson who really impressed upon Little that the way to get the money pouring in was to talk up *Mus musculus* as a way to investigate human medical conditions.

The new facility opened in Bar Harbor, Maine, in 1929. Jackson’s sudden death led it to be dubbed the Roscoe B. Jackson Memorial Laboratory—better known today as the Jackson Lab.

Little’s first ambition was that the lab be a venue for basic research, using mice. But the Depression, a decrease in funding from his auto executive backers, and slow research results all conspired to force the lab to sell samples of its stocks. And while the lab is certainly home to research, the development and sale of strains of so-called JAX® mice made it famous.

The lab supplies about two million individuals a year, representing some 2,800 varieties. Former director Kenneth Paigen estimates that 95 percent of all the model mice used in labs worldwide had their origins at Jackson. Whitehead Fellow Mark Daly notes that scientists can choose from no fewer than 114 Jackson varieties for diabetes-related research alone. And one of Little’s first stable strains, known throughout the research world as C57BL, was the mouse whose genome was sequenced.

History has shown that Little’s instincts were good ones. With fast generation times and large litters, mice are the best mammals for genetics research. And their sequence similarities with us—now pegged at about 99% based on the comparison of the two genomes—means that in most cases they also are the best mammalian subjects for medical research. Plus, their generally easy attitude about lab life makes them easy to work with. *Mus*, no fuss.



AFTER CHANGING THE RESEARCH LANDSCAPE FOR GENE EXPRESSION, MICROARRAYS ARE

Figuring what a gene does is hard work, but it's vastly easier than it was a few years ago. Back then, you would laboriously isolate a single gene, tinker with it to get some inkling about its purpose, and then start speculating about how it might collaborate with other genes. Now, microarrays let researchers gather exponentially more

robotic system for screening thousands of genes at once on small glass slides.

By fixing tiny dots or “probes” (each a short bit of DNA or RNA) onto a slide in an organized grid, scientists fashioned a microarray, also known as a gene chip or DNA chip. The dots were so tiny that a slide could hold thousands of probes.

Next, a sample solution of “target” RNA (actually a solution of DNA and RNA from broken cells) was dropped on top of the microarray slide and held by a slide coverslip.

Each probe now served as an individual test. When an RNA target came in contact with matching DNA on the slide, it stuck. The target was labeled with a fluorescent tag. The better the match, the more it glowed. Researchers could measure each reaction and discover which genes were active for a given target.

Just a year later, Affymetrix introduced the first commercial microarray. In 1997, the first complete eukaryotic genome on a microarray (baker's yeast) was published in *Science*. DNA

microarrays vaulted to the forefront of biotechnology, in turn spawning RNA-based arrays and protein-based arrays.

Researchers in Whitehead Associate Member David Sabatini's lab now are upping the ante with new cell-based microarrays that promise much for the field of drug research. Unlike traditional cell assays, these experiments can test thousands of living cells at once to study cell pathways and drug interactions, and let researchers examine how these pathways and drug interactions affect each other.

This cell-based microarray technology “has great potential for high-throughput screening,” says Norbert Perrimon, a Harvard Medical School investigator who studies gene functions on a genome-wide scale in the *Drosophila* fruit fly.

A GENOME ON FOUR SLIDES

The miniaturized aspect of microarrays is key to their allure. Cell-based microarrays will let the Sabatini lab test every gene in the entire fruit fly genome using just four glass slides and a relatively newly discovered phenomenon known as RNA interference (RNAi). The lab recently proved this in principle and is now working its way through the genome.

To use RNAi, scientists synthesize small RNA molecules to target and “knock down” a specific gene



Douglas Wheeler and David Sabatini are developing microarrays for tests with living cells. Top, closeup of such an array, with spots that are alive (blue) in a field of dead cells.

data about gene expression—what each of thousands of genes *does*, perhaps in coordination with other genes.

The microarray was born a decade ago as a solution to the one-gene-at-a-time dilemma. While studying the growth and development of *Arabidopsis thaliana*, the mustard plant, Stanford University scientists created a



Array for the cell

TURBOCHARGING STUDIES WITH LIVING CELLS BY JENNIFER TOMASE

by blocking its ability to create protein. Each RNAi molecule is specifically designed for its target gene, so a 20,000-gene genome, such as the fruit fly's, requires 20,000 RNAi molecules.

While these molecules can be bought or generated in the lab, the sheer volume required is a challenge. The Sabatini lab needs significantly fewer with its microarray format than it did with microtiter plates, a previous technology. On a microarray, each spot needs only enough RNAi molecules to "infect" about 300 cells. In contrast, a microtiter plate well holds many thousands of cells and needs a corresponding number of RNAi molecules. Also, microtiter plates hold 384 wells each. But microarray slides can hold thousands of samples—5,000 in this case.

Cells are then "seeded" on the array. A solution of cells and cell growth media is poured over the microarray slide in a Petri dish. After an hour or two, the cells land on the bottom of the dish and attach. A layer of cells grows over both slide and Petri dish. Cells that land on an RNA spot probe are affected by the probe and their reactions can be analyzed.

Plenty of groups have done RNAi arrays, notes Douglas Wheeler, a technical assistant in the Sabatini lab, but they've used mammalian cells. Those have serious technical drawbacks, including a response to interferon

proteins (which cells produce in reaction to viral infections) that makes cell uptake of the large RNAi molecules difficult.

Recognizing this, the Sabatini lab committed to the same work in *Drosophila*. Much of the genome is conserved between fruit flies and mammals, and the fly cells easily take up RNAi molecules. "Because *Drosophila* is so easy, why not do the initial throwing out of the net with a screen in *Drosophila*, and then do the follow-up in mammalian cells?" asks Wheeler.

This approach should allow scientists to pin down pathways for cellular growth, with implications for cancer research. "We could knock down every gene in the genome to find which genes affect how fast cells replicate and how big they get," says research assistant Steve Bailey.

FASTER DATA, FASTER DRUGS

In a second line of research, cell-based microarrays let researchers test chemical compounds on cells by the thousand. The more of these "small molecule" compounds tested, the better the chance of finding one worthy of further testing for therapeutic value. Cell-based microarrays, which connect live cells with possible drugs, can churn through large numbers of compounds.

In one such experiment, Bailey used a robot to print the same 72 com-

pounds onto a series of slides. To hold the compounds in place, he embedded them in a biodegradable polymer, creating a series of tiny parallel dots. "They actually look, at the microscopic level, like Braille," says Bailey.

Bailey then covered the slides with cells. The polymer slowly dissolved, and he measured how the cells reacted to the compounds. This technique, he suggests, could be applied to cells that mimic cancer cells. If you combine these cells with myriad compounds, and one compound in particular kills the cells, you might just have a potential drug to fight cancer. The lab hopes to license the technology.

The microarrays can do "double knockdowns," manipulating more than one gene at a time to see if and how they're connected. "One can very quickly go through very large numbers of genes and very large numbers of cell phenotypes and say what genes are responsible," says Sabatini.

"These microarrays allow use of rare cell types (because we only need a few cells per experiment), allow use of rare compounds, and allow imaging of cellular functions and molecules," adds Brent Stockwell, an assistant professor at Columbia University and former Whitehead Fellow who developed the microarrays jointly with Sabatini. "They should accelerate both basic biology and drug discovery efforts."

KNOCKOUT PUNCH

RNA interference is a breakthrough in the lab, but can it be turned into useful medicine?

DEEP IN YOUR DNA, A GENE HAS GONE HAYWIRE AND IS DRIVING UP the production of a protein that is messing with your body. Wouldn't it be great to sift through all your 20,000-something genes, find the offender, and swat it like a fly?

Fortunately, a new technique eventually could do just that, targeting that gene and *only* that gene, knocking it out of operation and relieving your distress with zero side effects.

That's the audacious theory, anyway, behind the medical application of RNA interference (RNAi).

When RNAi emerged on the research scene five years ago from experiments at Whitehead and other labs, it was hailed as a critical breakthrough for science. The approach involves delivering tiny strands of RNA into target cells. These strands interfere with the messenger RNA molecules that control protein production and hence gene expression, giving scientists the power to knock out individual genes at will.

Now a vital tool for genomic exploration, RNAi also promises to create new drugs that would target the genetic roots of disease.

Several classes of RNAi-based drugs are at advanced stages of development. One—a treatment for age-related macular degeneration (AMD) of the eye—is in Phase 1 clinical trials. Other RNAi-based drugs still in pre-clinical development target HIV, hepatitis C, Huntington disease, and various neurodegenerative disorders.

In the race toward the clinic, RNA is at a turning point. While scientists generally remain upbeat about its clinical potential, they also are still cautious in their views. Getting the tiny strands of RNA (known as short interfering RNAs, or siRNAs) into target cells is no easy task.



AIDS

Delivering RNAi compounds and ensuring that they last long enough to be useful pose continuing challenges. Some researchers doubt that RNAi will ever make it out of the lab, pointing to the decades of largely unsuccessful struggle to commercialize antisense DNA, another approach to selective gene silencing.

PREPARING FOR TRIAL

While working at Whitehead from 1999 to 2001, Whitehead Member David Bartel and colleagues who include Thomas Tuschl (now at Rockefeller University), Phillip Zamore (now at the University of Massachusetts Medical School), and Phillip Sharp (still at MIT) generated early insights into RNAi mechanisms in cell biology.

The group knew the research would be important for both fundamental and clinical research. “None of us had the resources or ability to

develop this as a clinical tool,” Bartel says. But as pioneers in the field, with a series of influential papers behind them, the scientists and their business colleagues had little trouble securing capital for a startup. Raising \$17 million, in 2002 they founded Alnylam Pharmaceuticals, which has since become a public company.

Headquartered a few blocks away from Whitehead, Alnylam focuses on diseases that are well understood genetically but have proved difficult to treat effectively with more conventional drugs. That list currently includes age-related macular degeneration (AMD, the leading cause of vision loss in the U.S.), respiratory syncytial virus (RSV), spinal cord injuries, Parkinson disease, and cystic fibrosis. Half of Alnylam’s 70-plus employees are MD or PhD researchers.

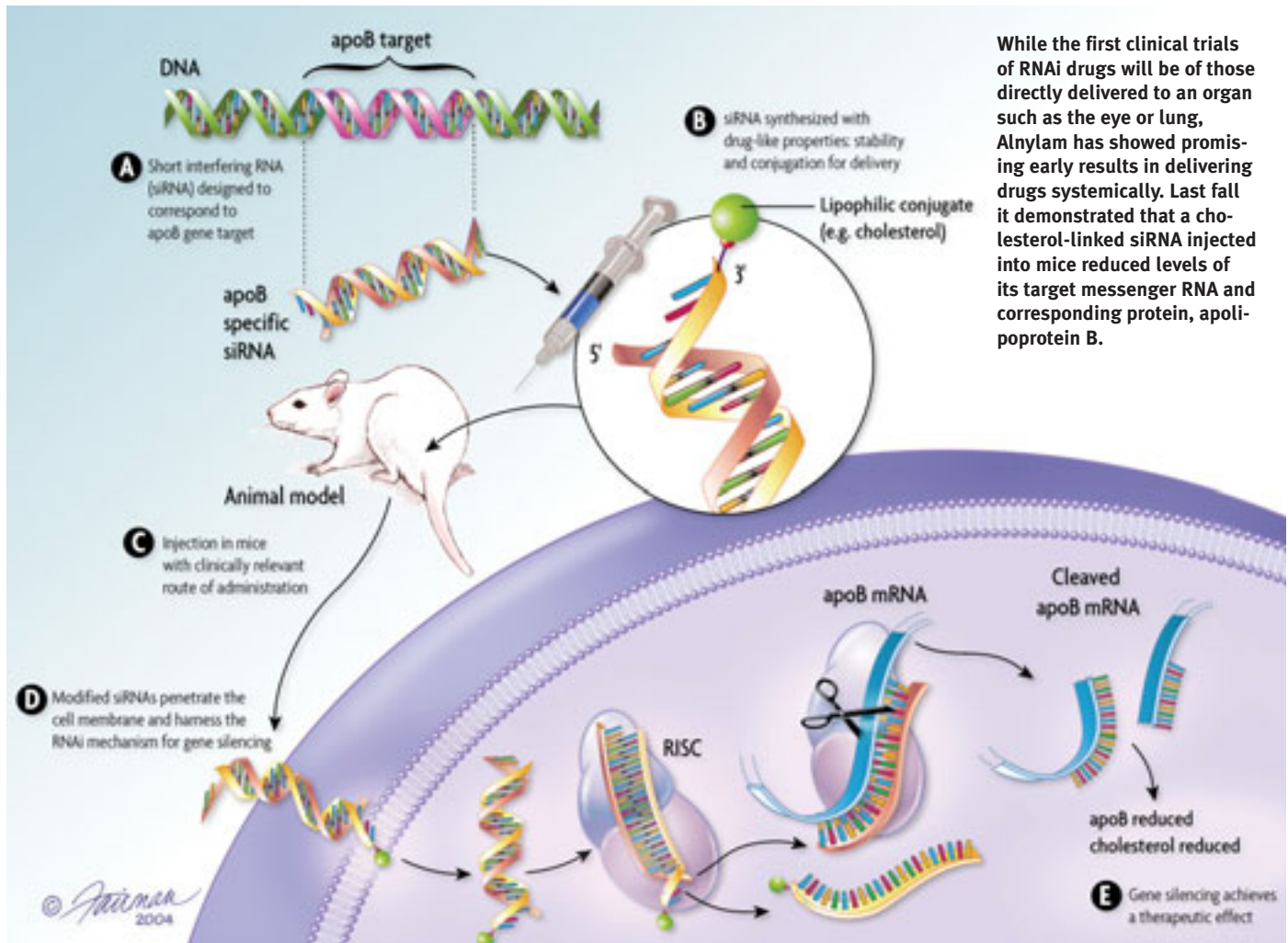
The company’s drug process kicks off by selecting a suitable disease, says

John Maraganore, president and chief executive.

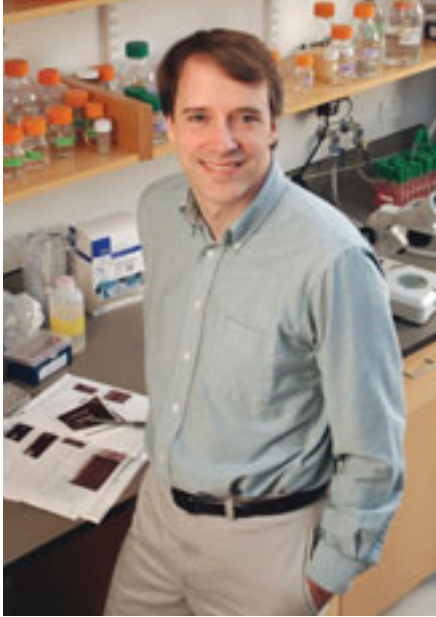
Second, researchers design and synthesize siRNAs that are predicted to be effective against the target. There might be 100 or 200 of these molecular candidates, with sequences chosen to work preferentially against the target gene’s messenger RNA. Another practical consideration is to choose candidate sequences that are identical between humans, mice and any other model organisms.

“Then we take the synthetic siRNAs and screen them in cell-based arrays to target reductions in messenger RNAs and proteins,” Maraganore says. “We can test a couple of hundred siRNAs within a week or so. It’s pretty common that we find 10 to 20% of them that are much more potent than the others.”

Next, the researchers screen out siRNAs that might activate the inter-



While the first clinical trials of RNAi drugs will be of those directly delivered to an organ such as the eye or lung, Alnylam has showed promising early results in delivering drugs systemically. Last fall it demonstrated that a cholesterol-linked siRNA injected into mice reduced levels of its target messenger RNA and corresponding protein, apolipoprotein B.



David Bartel, Whitehead Member and Alnylam co-founder, played a key role in early work on RNA interference.

feron reaction in cells. (RNAi molecules that look like viruses to the cell can risk being wiped out by interferon proteins that cells produce and release to the bloodstream in response to viral invasions.) Then the researchers modify siRNAs chemically to enhance their ability to pass through the cell membrane and withstand attack by enzymes within the cell. Now the survivors are ready for toxicology studies in animals, the final step before human trials.

Alnylam expects that its AMD candidate will reach human clinical trials by the end of this year, followed by its RSV drug. The pre-clinical development cycle is surprisingly fast compared to that of small-molecule or protein drug discovery, since it starts with such carefully honed targets, Maraganore says. And while human trials will follow the same procedures

and schedules that they do with more conventional drugs, he hopes that success rates will be higher since RNAi follows natural pathways.

THE VISION THING

Here's a similar startup story: During the late 1990s, Michael Tolentino, an ophthalmologist at the University of Pennsylvania, and colleague Sam Reich found that RNAi effectively silenced genes in the mammalian eye. The scientists proposed that RNAi could offer new treatments for AMD, a condition that occurs when the vascular endothelial growth factor (VEGF) protein becomes hyperactivated. This protein contributes to the growth of abnormal blood vessels in the eye, which leak and produce dim central vision in millions of aging patients. Other treatments have offered only limited success. Reich licensed the technology from the university, and Acuity Pharmaceuticals of Philadelphia was created in 2002.

The company's drug development efforts have been based largely on the initial research by Reich and Tolentino. Reich—who serves as Acuity's vice president for research and development—staffed the company with experts in basic research, manufacturing and regulatory affairs.

Acuity president Dale Pfof says that company researchers built on the university experiments with a series of in vitro and in vivo experiments designed to screen for potential drug candidates. These efforts eventually led to the isolation of Cand5, an RNAi

compound that is now the company's chief product.

The firm's scientists then moved on to a series of pre-clinical studies designed to evaluate the drug's absorption, distribution, metabolism and excretion in a range of mammalian species. These data, gathered in-house and by contract laboratories, must be submitted with an investigational new drug application filed with the Food and Drug Administration.

Currently Acuity is the only company with an RNAi product in clinical trials. Phase 1 clinical trials with Cand5 began in late 2004. Pfof predicts the drug will be approved by the FDA and on the market by 2009.

SPECIAL DELIVERIES

AMD is likely to be the first human illness for which RNAi yields approved treatments, says Irena Melnikova, a senior research analyst with Life Science Insights, a subsidiary of IDC Research. That's because RNAi compounds can be injected directly into the eye, avoiding the systemic barriers that plague effective delivery of the compounds elsewhere in the body. Several companies are now working their own angles on the disease.

Acuity's closest rival, Sirna Therapeutics of Boulder, Colorado, has an RNA compound called Sirna-027 that targets a VEGF receptor protein rather than VEGF itself.

Compound delivery methods also differ. While Sirna-027 is unmodified from its natural state, Cand5 is chemically modified to enhance its stability.

THE DRUG DEVELOPMENT MARATHON

Development of a new drug typically takes a decade or more at a cost approaching a billion dollars, according to the Pharmaceutical Research and Manufacturers Association of America. Here are the steps and representative time frames. While a radical new approach

such as RNA interference may add even more uncertainty to this lengthy process, proponents say that pre-clinical testing may end up being quicker than for more traditional drugs. That's because RNAi work can exploit new genomic knowledge and high-throughput technologies to target genes whose role in disease is well understood.

PRE-CLINICAL TESTING	PHASE 1 CLINICAL TRIALS	PHASE 2 CLINICAL TRIALS	PHASE 3 CLINICAL TRIALS	FEDERAL DRUG ADMINISTRATION APPROVAL
3-6 YEARS	1-2 YEARS	2-3 YEARS	3 YEARS	2-3 YEARS
Assess safety and biological activity	Determine safety and dosage	Evaluate effectiveness and side effects	Further evaluate effectiveness, monitor long-term use	Review for approved use
Laboratory and animal studies	20 to 80 healthy human volunteers	100 to 300 patient volunteers	1,000 to 3,000 patient volunteers	

Another class of RNAi-based drugs now on the verge of clinical trials operates through a different approach often described as gene therapy—or as “expressed RNAi” because it harnesses the cell’s own genetic machinery to produce a gene-silencing response. Typically, a viral vector (viral DNA modified to carry the desired DNA) transfers instructions for making RNAi molecules directly into the cell’s genome. The cell then produces the molecules as part of a natural process.

One potential treatment based on the expressed approach targets HIV. Benitec in Mountain View, California, is creating a drug that targets a set of three genes involved in HIV. Among them, a “master regulator” called TAT controls other genes in the virus.

John Rossi, who chairs Benitec’s science advisory board, is a professor at the Beckham Research Institute at the City of Hope cancer center in Duarte, California. “We feel that with an RNAi cocktail that targets three genes, we’ll be able to keep HIV in check,” he says. Rossi, who previously worked on antisense strategies for gene silencing, is quite keen on the potential of RNAi. Benitec plans to take its compound into Phase 1 clinical trials this November.

YELLOW LIGHTS?

Some researchers suggest that opportunities with RNAi may never rise beyond basic research. At best, these skeptics say, RNAi screening will speed up identification of proteins that can be better targeted with standard drugs.

Part of this skepticism comes from disappointments with previous attempts at selective gene control. For more than a decade, researchers have struggled to create successful therapies with antisense drugs that also bind to messenger RNA. Only one such drug is in use, treating certain eye infections in AIDS patients.

One issue is that antisense drugs tend to degrade rapidly, so their potency is low. RNAi proponents say that RNAi-based preparations are up to 1,000 times more active than their antisense counterparts, indicating a vastly greater likelihood of therapeutic success.

For RNAi, systemic delivery to target cells in the body remains a huge obstacle. Researchers are focusing on ways to bypass cell membranes and evade immune responses that might degrade the drugs too soon.

Alnylam made headlines with a paper, published in *Nature* last



Lubomir Nechev oversees the synthesis of siRNA molecules at Alnylam Pharmaceuticals, which plans its first clinical trial this year.

November, showing that RNAi compounds administered by injection could silence clinically relevant genes if they were attached to cholesterol molecules. The work demonstrated for the first time that gene silencing could be achieved in live animals through a systemic route of administration.

But dose levels were extremely high and yielded only a partial effect. The company is working to design a compound that goes directly to the target tissue and is more easily taken up.

INTO THE PHARM LEAGUES

For RNAi companies to succeed, they must get the blessing of major pharmaceutical companies. For the time being, says Sara Cunningham, Benitec chief executive, the major pharma companies are on the sidelines, waiting for safety and efficacy data to emerge from pre-clinical and Phase 1 research.

In the meantime, RNAi researchers are running as fast as they can.

“RNAi has enormous potential as a therapy,” says Judy Lieberman, an RNAi researcher who teaches pediatrics at Harvard Medical School.

“It’s hard to predict at this stage if it’s going to be as promising as some people might think,” sums up Lieberman. “But it’s extremely active at very low concentrations with a high degree of specificity. I think it can be used with almost any gene, so the disease opportunities are pretty unlimited. I’m very optimistic.”



The free-for-all over RNAi intellectual property rights probably will end with a flood of cross-licensing, predicts Benitec’s Sara Cunningham.

Patent war—and peace

Intellectual property is the name of the game in RNAi-based medicine. Companies that can defend their positions with strong claims to novel research will control how the research turns into solid business opportunities.

More than 900 patent license applications for RNAi technology have been filed in the U.S. Only two patents have been issued.

That means research is advancing in a Wild West atmosphere, where companies stake out claims to intellectual property that they then strive to defend from competitors. Because these claims are all based on patent applications—which offer no actual protection—companies are free to

criticize each other’s positions, which they do with unfettered zeal. “There’s a lot of mudslinging going on out there,” says Benitec CEO Sara Cunningham.

But patent debates probably won’t block efforts to get a product on the market, she predicts. Most biotechnology companies are too small to pursue protections that actually exclude competitor access. The more likely scenario, Cunningham suggests, is one in which firms issue licenses to their own technology so they can capitalize on the success of the competition. “Which is fine because that’s how biotechnology tends to work,” she says. “It tends to be cooperative.”



The double life of Christopher Hug

Splitting their time between lab and clinic,
Whitehead physician-scientists bring research and reassurance to patients

BY CAROL CRUZAN MORTON | PHOTOGRAPHS BY KATHLEEN DOOHER

BREATHING CAN GET A little competitive in the fourth-floor pulmonary clinic at Children's Hospital in Boston.

In an exam room, a lanky 15-year-old boy, his lips around a plastic nozzle, sucks air through clear plastic tubing hooked up to a laptop computer.

"Deep breath," coaches the pulmonary-function technician, watching the air flow measurements on the computer screen. "Bigger, bigger, *bigger!*" A pause. "Push, push, push, *push!*" The boy exhales every last bit of air, red in the face.

Test over, the boy draws a normal breath and immediately doubles over in a fit of thick coughing. He recovers, and checks out his scores on the screen. Then he convinces the technician to repeat the test and try for a better result, as if it were a fifth attempt on a computer game.

His doctor talks to the boy's mother and looks over the test results from last year. The cold end of a stethoscope draped around the doctor's neck partially obscures the blue cursive stitching on the white lab coat that spells out "Christopher Hug, MD, PhD."

"You would be hard pressed to say he has cystic fibrosis, looking at these curves," says Hug, pointing out how closely the graphed air volume and velocity match those of an average healthy teenage boy.

Some people with cystic fibrosis, the most common lethal inherited disease, still die in their teens. But better nutrition, antibiotics and mucous-clearing medicines have helped many live well into their 50s and 60s. Yet for all the advances in understanding the genetic mutations and molecular mechanisms of the disease, a cure is still elusive, and a lung transplant remains inevitable.

Nearly every day, researchers announce important new discoveries with the potential to alleviate much human suffering. In fact, advances in basic science are piling up faster than other researchers can figure out how to apply that knowledge to disease.



"In the clinic, where you are managing chronic illnesses, you can lose sight of the bigger picture of how to prevent or cure diseases." —Christopher Hug

The future of medicine depends upon physician researchers like Hug to close that gap. "In order for medicine to progress there is need for physician-scientists who understand clinical medicine and for basic scientists who can effectively communicate and collaborate with them," said Irwin Arias of the Tufts University School of Medicine in a report published last year by the National Research Council, "Bridging the Bed-Bench Gap."

BETWEEN BIO AND MEDICINE

Hug, a postdoctoral fellow in the Whitehead laboratory of Harvey Lodish, is in the final stages of training for a career designed to bridge the two worlds. It has been a long haul. Hug graduated from college 18 years ago, and at age 39 looks forward to establishing his own lab soon, while continuing to consult with patients.

In the lab, where Hug spends most of his time these days, the typically tedious, laborious and faltering pace of

research can be disheartening. In the hospital, where Hug spends Mondays in the outpatient clinic and four weeks a year in the inpatient wards, it can be heartbreaking.

Down the hall, an anguished father asks Hug if his 17-year-old son, paralyzed by a mysterious infection of the spinal cord three years ago, can sign up for the stem cell experiments that were supposed to save Christopher Reeve. The boy can wiggle his toes and move his hands enough to spin his wheelchair in playful circles. Today he breathed for 15 minutes on his own without his ventilator. But healing has been slow, and stem cell research is still far from offering even an experimental option.

“In the clinic, where you are managing chronic illness, you can lose sight of the bigger picture of how to prevent or cure disease,” says Hug. “In the lab, it’s good to be motivated by clinical work.”

Hug ducks out of the examining room to renew the usual medications and order the appropriate lab tests.

The science behind the prescriptions he writes reaches back more than 60 years. In 1943, a Danish and a U.S. biochemist shared a Nobel Prize for the discovery that vitamin K, named for the Danish spelling of “coagulation” (“koagulation”), could prevent severe internal hemorrhaging in chicks.

Antibiotics for the frequently life-

threatening lung infections in people with cystic fibrosis also kill the bacteria in their guts that produce about half the daily supply of the nutrient. Low levels of vitamin K, combined with lung damage, can lead to lethal pulmonary bleeding, Hug says.

To complicate matters, people with CF have trouble digesting dietary fat, including the fat-soluble vitamins A, D, E and K. So Hug also prescribes pancreatic enzymes to help their bodies absorb the supplements.

Another medication, DNase, is a more recent product of modern science. Discovered by veterinarians in the 1970s and rediscovered by medical researchers in the 1980s, DNase became the first new drug for the management of CF in 30 years when it was launched by Genentech in 1994. Delivered through an inhaler, it cleaves the DNA of dying cells in the lungs of people with CF, helping to thin the thick mucous that builds up in the airways. Respiratory failure accounts for about 90% of CF deaths.

The scientist in Hug has idly wondered how DNase could be so effective. In his graduate research, Hug used DNase in test tube experiments to bind to and count actin filaments, the infrastructure of cells in most organisms. Actin, which is also present in the dying lung cells of people with CF, can interfere with the ability of DNase to degrade DNA.

But now, the doctor’s chief concern is that the boy take the necessary 15 minutes every morning to use the aerosol device that delivers the life-extending drug. This and other medicines to fight inflammation and help clear the mucous can buy decades of life. A lung transplant can promise only two to five extra years for the 50% of patients who survive the operation. “He’s used to coughing,” Hug points out. “He may not appreciate that [the medication] will slow down the decline in lung function we know will happen with time.”

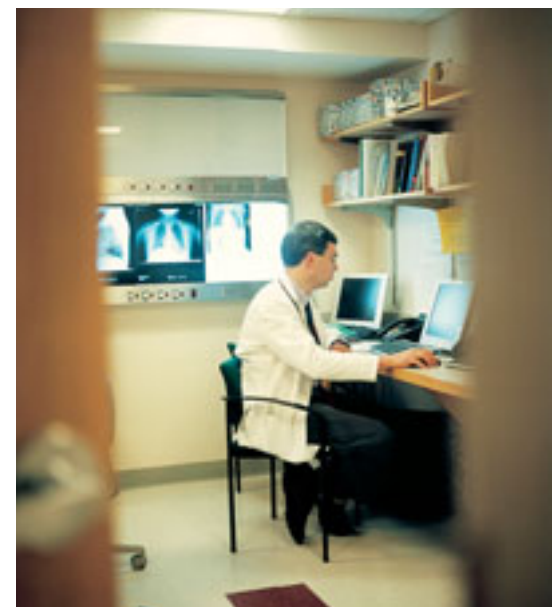
Hug finishes the scripts and grabs the notes on his next two patients, a four-year-old boy whose asthma flare-up resolved itself in the long time it took to get an appointment at the

clinic, and a 15-year-old whose mother was worried about a pain in his chest that a cardiologist had ruled out as a heart problem.

FIELDS OF FOCUS

Doctors and scientists are trained to approach biomedical problems in very different ways, observes Bradley Bernstein, MD, PhD, a pathologist at the Brigham and Women’s Hospital in Boston and the recipient of a Howard Hughes Medical Institute physician postdoctoral fellowship in a chemistry lab at Harvard University.

“In medicine, it’s breadth,” Bernstein says. “You need to know a little about everything. It’s completely the



opposite in science. You need to be the world’s expert on an incredibly narrow area.”

A day in the clinic varies from a day in the lab in other ways, says pediatric neurologist Annapurna Poduri, MD. She specializes in childhood epilepsy and is spending the year in a neurobiology laboratory at Boston’s Beth Israel Deaconess Medical Center testing the hypothesis that localized genetic changes result in the brain malformations that are observed in many patients with epilepsy.

“The clinic is more structured,” Poduri says. “You have skills, an acknowledged competence, immediate feedback from your patients, and a



Filling the bench-to-bed gap

“Since the early 1970s, the gap between basic science and medicine has increased largely because science has become more complicated,” Irwin Arias of the Tufts University School of Medicine wrote in a report last year. “Physicians are not entering patient-oriented research at a time that provides the greatest opportunities for research into the cause, mechanism, prevention and treatment of major diseases.”

Nationwide, there is room for about 2% of first-year medical students in the medical scientist training programs leading to the type of combined MD/PhD degree held by Christopher Hug.

And medical students are not exactly pounding on the doors of labs. “Only 6% of first-year matriculants think curing disease is the most important purpose of medicine,” noted Eric Neilson of Vanderbilt University School of Medicine last year in the *Journal of Clinical Investigation*. “The average person on the street seems more committed to medical research than our students.”

Other factors discouraging a dual career include the prolonged training, the burden of keeping up in two fast-moving professions, the competitiveness for research funding, the pressure for doctors to see more patients, and the time and motivation to do both.

The National Institutes of Health offers funding specifically for patient-oriented research for investigators at the beginning and middle of their careers. NIH also has a program to pay off the large debts of recently graduated doctors who conduct patient-oriented research.

The Howard Hughes Medical Institute launched a \$10 million program in December that awards grants to science graduate programs that incorporate medicine and pathophysiology. HHMI already supports two programs that introduce medical students to basic research. Other private foundations also provide support to physician-scientists, such as the Charles H. Hood Foundation Child Health Research grant that funds Hug’s work. And the pediatric pulmonary division of Children’s chips in as well.

sense of closure at the end of the day. In the lab, though, while you have the freedom to organize your own time and design your own projects, you are working without knowing the results of your experiments for long periods of time. You may not have a sense of conclusion for weeks or months.”

Poduri and Hug met during their residency training at Children’s Hospital and married two years ago. Coincidentally, Hug’s father and mother also met and married when both were working at Children’s Hospital 45 years ago.

On top of the challenges of a double career in science and medicine, physician-scientists also may be juggling



the demands of dual-career families. “Many MD/PhDs marry another MD or PhD, because that’s the only type of people you see when you’re training,” says Robert Flaumenhaft, an assistant professor in hematology at Beth Israel Deaconess.

Flaumenhaft is married to a clinical endocrinologist. When it is time to pick up the kids from school, they call each other to determine who is more desperately behind in his or her work. Because Flaumenhaft is concentrating on his research these days, it is often easier for him to leave his test tubes than for his wife to leave her patients.

One day, stuck in traffic on the way to the zoo, with her parents driv-

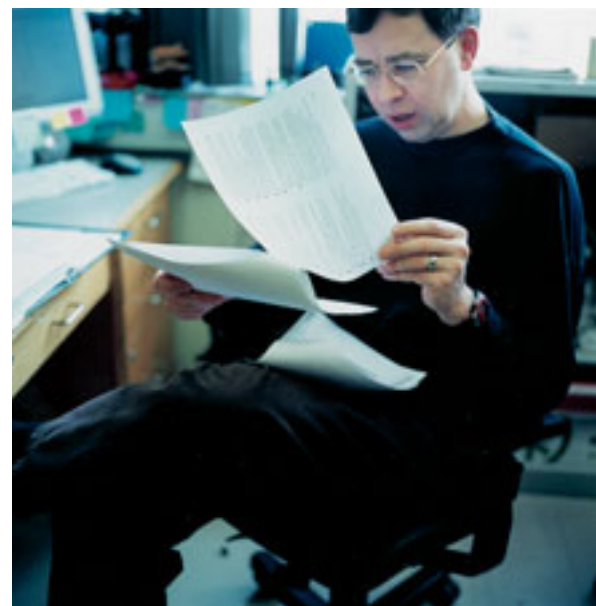
ing and their three children sitting on their parents’ laps in the backseat, Flaumenhaft and his wife came up with an idea to collaborate on a research project to analyze molecular signaling in the blood platelets of patients with diabetes. The grant for the project was funded.

LIVES OF A POST-DOCTOR

It is a light morning in the pulmonary clinic. Hug’s last patient ends the morning on an upbeat note. The energetic four-year-old was born prematurely at 23 weeks weighing only one pound, his mother explains. Today, the scale reads 34 pounds with clothes. He breathes enthusiastically while Hug listens with his stethoscope. The boy’s abdomen shows the scars of several tubes that once sustained his life when he was an infant. The lungs sound good, and Hug prescribes a different dosing regimen in anticipation of weaning him off his lung medication soon.

“When you pick up a baby, there is a strong, hands-on incentive to study the problem,” Hug says.

He rushes downstairs to scavenge leftovers and catch the end of the lunchtime talk given today by Poduri. After lunch, Poduri and Hug compare their schedules. Hug has an hour of dictation ahead of him. Then he will meet with an asthma researcher to discuss a basic science project that may





“In the lab, it’s good to be motivated by clinical work.”

tie together Hug’s pulmonary clinical expertise with his Whitehead research on molecular signaling in fat cells, by way of related inflammatory processes.

Hug first met Harvey Lodish at a pool party hosted by Lodish’s daughter, a fellow resident-in-training at Children’s. Two years later, he chose the Lodish lab for his postdoctoral fellowship because he wanted to work with a preeminent cell biologist.

His research is on adiponectin, a hormone released by fat that was

discovered in the Lodish lab a decade ago (see “Fat chance” on page 20). Since then, researchers have linked low levels of the hormone and the location of its genes to cardiovascular disease, diabetes, obesity, high blood lipids and hypertension. Large doses of the protein can reverse insulin resistance in mice and cause obese mice to lose weight.

Last June, Hug reported discovery of a receptor for the protein, located on cell surfaces in blood vessels in the

heart and muscle tissue. He continues to search for other receptors that will help scientists understand how the hormone works and how to develop a molecule that can mimic its protective effects in patients.

At this stage, there is little connection between Hug’s basic science and his clinical work, but Hug is confident that his life in the clinic and the lab will converge.

After all, making novel connections is a main point of the dual MD/PhD training, says Howard Hughes investigator Daniel Goldberg, MD, PhD. Goldberg heads the country’s largest physician-scientist training program at Washington University Medical School in St. Louis, from which Hug graduated nine years ago.

While there is a cultural gap between doctors and scientists, “painting it in black and white is an exaggeration,” Goldberg says. “Some people can move between them, even if their ways of thinking are different. The best clinicians don’t just treat the disease; they treat the patients. PhDs who do not have a full appreciation for the human body and everything that can go wrong may not realize when something comes up in research that could be important medically.”

A handful of other Whitehead researchers also are bridging this gap. Several of these work at the Lodish lab, which has a long history with clinician/researchers. They include Aleksandar Babic, a clinical pathology resident at Brigham and Women’s Hospital; Shilpa Hattangadi, a pediatric hematology oncology fellow at the Dana Farber Cancer Institute and at Children’s; and Andreas Herrlich, a renal fellow at Massachusetts General Hospital.

Tonight, Hug will be back among them at Whitehead, working with the lab’s fluorescence-activated cell sorter, which uses light to sort cells based on their size and color.

“If I work as fast as I can in the clinic, I can help 20 patients a day,” Hug says. “But if I learn something in the lab that can be used to develop a new treatment, it will help hundreds of thousands of patients.”

FAT C H A N G E



Better understanding of fat-cell hormones will help us attack the twin epidemics of obesity and diabetes

BY DAVID CAMERON | Photograph by Sam Ogden

IMAGINE THIS public-health drama as a film with two parallel plot lines.

Here's the first plot line: The U.S. has a problem, a *big* problem. We're increasingly becoming a nation of overweight—and often downright obese—people. Just look at the numbers. Statistics from 1985 show that less than 10% of the populations of New York and California qualified as being obese. Fast forward to 2001, and that number jumps all the way to 24%.

The second plot line unravels another drama: The onslaught of type 2 diabetes. This disease, afflicting nearly 10% of adults and a rapidly increasing

number of children, is the leading cause of blindness and kidney failure, and ranks sixth on the list of killers. And the number of cases is soaring.

You've probably guessed the first plot twist: Both stories star the same villain.

While obesity plays a big role in other diseases ranging from cardiovascular disease to cancer, it tracks particularly well with the epidemic of diabetes. The state of Mississippi offers one telling example. While the rates of obesity there climbed from ten percent to over 25% during the 1990s, rates of type 2 diabetes climbed from 6% to over 10%.



When Harvey Lodish's lab demonstrated how adiponectin burned off fat in mice, journal reviewers "simply didn't believe it."

that transports this sugar across cell membranes. He also has been a leader in studying the hormones that fat cells secrete, comments diabetes researcher Jeffrey Flier of Harvard University.

The Lodish lab has helped to reveal why obesity is not only so toxic to an organism but also why it is so correlated with type 2 diabetes.

SHUTTLING SUGAR

Not all diabetes is related to obesity. Type 1 diabetes, often referred to as juvenile diabetes, has nothing to do with body weight. Rather, it's a condition in which the immune system attacks the insulin-producing cells in the pancreas, causing blood sugar levels to skyrocket. In type 2 diabetes, often called adult-onset diabetes, the cells in muscle and fat tissue start becoming resistant over time to the signals that insulin sends. Once again, blood sugar levels skyrocket.

It's been known for about 80 years that insulin is the hormone chiefly responsible for regulating glucose, the sugar in your blood that gives you energy. In fact, insulin is the first biotechnology product ever, manufactured in the 1920s to treat type 1 diabetes.

"Basically, insulin is part of a regulatory circuit," says Lodish, much of whose work has focused on the exact signaling process by which insulin communicates with cells.

Here's how it works:

Whenever you eat or drink, your digestive system releases glucose into your bloodstream. But the glucose can't make it into your cells without help from insulin.

As the glucose level in your blood mounts, certain cells in your pancreas start producing insulin and releasing it into your bloodstream. The insulin molecules make their way to muscle cells, where they bind to receptor proteins on the surface and send signals to proteins deep in the cytoplasm called glucose transporters—a class of proteins that was first identified in Lodish's lab in 1985.

Insulin lets these proteins know that there is a crowd of glucose molecules outside the cell eager to get in. The glucose transporters wake up from their cytoplasmic slumber and travel to the cell surface. Here, they merge into the cell surface membrane and morph into a kind of trap door, allowing individual glucose molecules to pass through one at a time into the cell.

But in obese people, this entire process begins to break down. "The insulin signal is sent, but the transporters respond sluggishly," says Lodish. "Eventually, they barely respond at all."

If glucose stays at high levels in the blood, diabetes sets in, with all its nasty complications. As Lodish describes it, though, type 2 diabetes is not so much a disease like lung cancer or Parkinson's. Rather, it's a cluster of symptoms that in many cases can be eliminated through diet and exercise. When the symptoms vanish, the person is technically no longer a diabetic.

So the key to understanding this condition is located squarely in the plump center of the fat cell.

SIZE MATTERS

For many years, fat cells were seen simply as cells that were. . .well. . .*fat*. That is, nothing more than passive repositories of triglycerides, the chief component of fats and oils. But that assumption took a hit in 1994 when Rockefeller University researcher Jeffrey Friedman discovered a hormone called leptin.

Studying mice that were genetically modified to be obese, Friedman found that leptin acts as a sort of thermostat for fat. When the mice ate too much, fat cells released leptin into the brain, where it would release a series of signals dictating that enough's enough.

Further studies showed that mice who were deficient in leptin couldn't stop eating and would thus become grossly obese. Once they were administered the hormone intravenously, their appetites returned to normal.

As it turns out, a small percentage of people are leptin-deficient and can be treated the same way. Undoubtedly, leptin is a "good" hormone.

But here's the real kicker: Friedman

In a Supersized nation where Burger King's latest breakfast sandwich weighs in at 730 calories and 47 grams of fat, more and more people have trouble squeezing their stomachs behind the steering wheel as they head over to the drive-through.

But the plot thickens yet again: Advances in medical understanding of the biology of obesity offer hope for a happy ending.

As these twin epidemics explode, so has our knowledge of the key molecules and mechanisms responsible for giving fat a bad name—findings that many pharma companies are now trying to exploit with anti-obesity therapies.

Harvey Lodish, a Founding Whitehead Member and professor of biology at MIT, has pioneered this field. Lodish opened up the field of glucose transport regulation by cloning the first protein

had found that leptin was produced by fat cells. So fat cells were no longer seen as inert units for triglyceride storage. They were active players secreting important metabolic hormones.

The following year, the Lodish lab made an equally startling discovery when it found another fat-cell-secreted hormone called adiponectin, which acts in concert with insulin in helping the cells to absorb glucose from the blood. Adiponectin also helps the body to burn off fat and sugar by stimulating the same chemical pathway that is activated when we exercise.

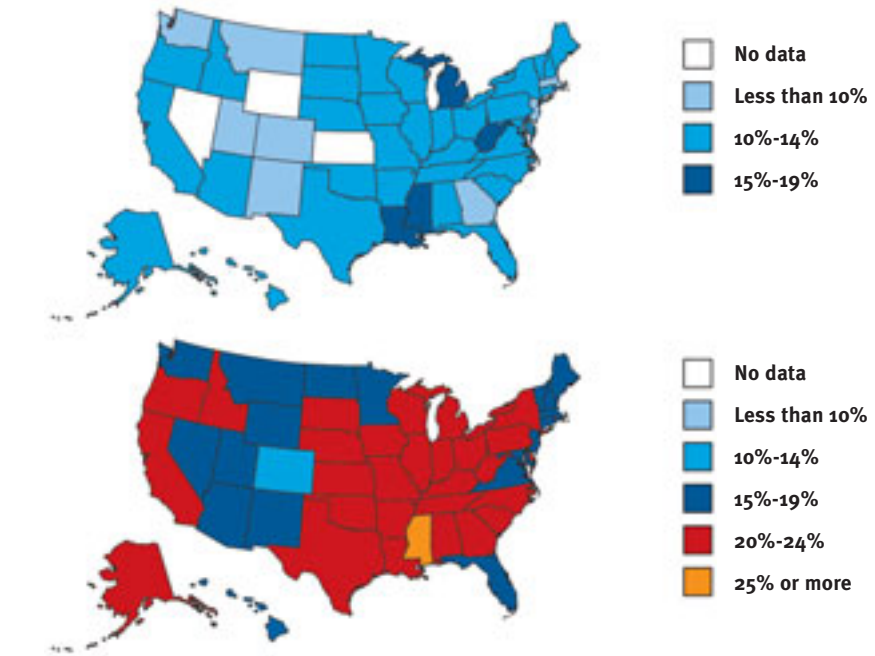
“Clearly,” says Lodish, “adiponectin is a good thing to have.”

The real power of adiponectin became clear in a paper that Lodish and co-workers published in the journal *Proceedings from the National Academy of Sciences* in 2001. Here, the researchers studied a group of mice that had been made obese through what Lodish refers to as a “cafeteria diet.”

A cafeteria diet is exactly what it sounds like. These mice were fed all the butter and sugar they wanted—and their desire knew no limit.

Once the mice were suitably obese, Lodish and his team injected them with adiponectin. The mice in turn increased their “burning” of the stored fat and lost weight—results that appeared to be almost miraculous.

“We tried to publish this in one of the major journals but couldn’t because the reviewers simply didn’t believe it,” Lodish recalls. “The fact that inject-



SUPERSIZED NATION

In 1991 (top) only a small handful of states, shown in dark blue, had an obesity problem affecting nearly 20% of their populations, according to statistics from the Centers for Disease Control and Prevention. By 2001 (bottom) in most states more than 20% of the population was obese. Rates of type 2 diabetes have followed a similarly disturbing trend.

ing adiponectin caused these mice to increase the burning of fatty acids was just too startling. Once we got it published I had to keep reminding the media over and over again how mice aren’t people. Many scientists didn’t believe our work until it was confirmed by two other labs the following fall.”

Adiponectin is a great hormone to have in abundance, and it’s a terrible hormone to lack. Rare genetic conditions that cause adiponectin deficiency may cause diabetes and heart trouble.

But here’s where things get counterintuitive.

If leptin and adiponectin are manufactured by fat cells, and if having them in abundance is beneficial, then doesn’t it stand to reason that the bigger you are, the more of these hormones you produce, and thus the healthier you should be? Isn’t there some kind of bigness benefit?

Before you reach for those Super-sized fries, the answer is no.

As it turns out, obese people

Six milestones in our knowledge of diabetes

- In 1869, the German scientist Paul Langerhans described “islands” of cells in the pancreas, which produce insulin and other hormones.
- In 1922, Frederick Banting and Charles Best at the University of Toronto discovered insulin by tying string around the pancreatic ducts of dogs and then, several weeks later, isolating the proteins left over in the pancreatic islets.
- Scientists in mid-century begin to suspect that there are two distinct kinds of

diabetes, one that typically begins in childhood, and another far more common kind that tends to originate with overweight adults.

- In 1959, Solomon Berson and Rosalyn Yalow at Veterans Administration Hospital in the Bronx developed the radioimmunoassay, a technique by which scientists can measure hormones in the blood. For the first time, doctors could now determine insulin levels in their patients. This led to clear understand-

ing of the differences between type 1 and type 2 diabetes.

- In the late 1960s and early 1970s, scientists began to realize that obesity produces a state of insulin resistance, a pre-diabetes condition.
- In 1994, Jeffrey Friedman of Rockefeller University discovered the hormone leptin, leading researchers to the conclusion that products of fat cells are important modulators of insulin activity.

become resistant to leptin. What's more, fat cells in obese tissue start to underproduce adiponectin, so obese people become deficient in this crucial hormone.

INFLAMMATORY NEWS

Now things get worse. Recent studies comparing fat tissue from normal-weight people and from obese people have provided further evidence that not all fat cells are created equal.

In people with normal weight, fat tissue contains precisely what you'd expect to find: lots of fat cells (known as "adipocytes" in the scientific parlance). But in obese people, fat tissue is loaded with cells called macrophages, cells that normally ingest pathogens and other foreign materials. When they ingest these foreign objects, they release inflammatory hormones that alert the immune system, hormones such as macrophage-produced tumor necrosis factor alpha (TNF α), a hormone that is elevated in arthritis and is also related to cancer and other conditions.

This makes perfect sense, because obesity is essentially an inflammatory disease, comments Gökhan Hotamisligil, professor of genetics and metabolism at the Harvard School of Public Health. "Excess calories affect the fat cells in such a way that they mount an immune response," he says. "You're activating the immune system without a legitimate pathogen," Hotamisligil continues. "You're constantly activating your immune system at a low level in such a way that it releases chemicals that start contributing to inflammation."

Obesity, then, causes stress, which alerts the immune system, which leads to the production of inflammatory mediators that interfere with the function of other metabolic pathways, which in turn causes stress.

"It soon turns into a vicious cycle," says Hotamisligil.

Lodish points out that the inflammatory hormone TNF α , which is found abundantly in fat tissue from obese people, blocks the expression of many fat cell genes that are vital for insulin action, including adiponectin (this is why obese people have less adiponectin in their blood).

Hong Ruan, a postdoctoral researcher in Lodish's lab, found that high levels of TNF α alter gene expression in such a way that fewer fatty acids are stored in the fat cells. Instead they are released into the blood, creating insulin resistance in the muscle.

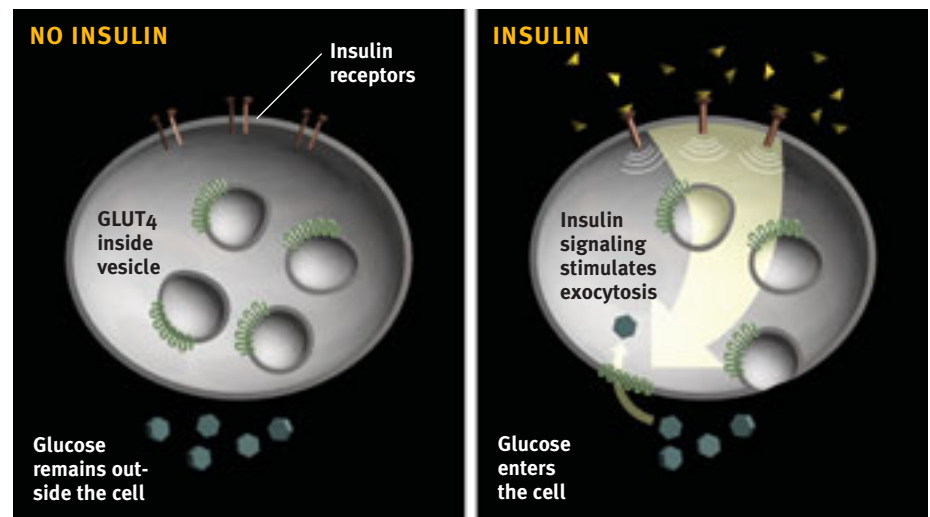
"This process goes on for many years, so eventually you wind up with low levels of adiponectin, high levels of fatty acids in the blood, and high levels of glucose in the blood," says Lodish.

But how might all these new

weight simply by taking your medicine.

"We've recently discovered seven other molecules in the genome that work the same way as adiponectin," adds Lodish. He just signed a licensing agreement with Wyeth Pharmaceuticals to work on these hormones.

The research joins hundreds of other projects shooting for weight-reduction drugs. And even though adiponectin activates the very same metabolic pathways stimulated by exercise, it probably won't be a chocoholic's dream come



HOW INSULIN AIDS GLUCOSE ABSORPTION

When no insulin is present, insulin receptors on the cell surface are inactive, and glucose (shown in gray) remains in the blood. But when insulin enters the bloodstream, the insulin receptors activate signals within the cell that allow glucose to enter via transporters such as GLUT4.

insights into the biology of obesity lead toward therapies?

OF MICE AND MEDICINE

Hanging on the wall of Lodish's office, near copies of the bestselling molecular biology textbook he co-authored, is Whitehead's patent on the hormone adiponectin, the molecule responsible for making those obese cafeteria-diet mice lean and mean.

While Lodish may be a hero to the world's millions of rodents, the hormone has yet to work the same kind of magic in people. Serono, the world's largest biotech, ended up acquiring rights to the molecule. And it's apparently hard at work trying to develop an adiponectin product that can be injected into people—perhaps the closest we could ever come to realizing every couch potato's fantasy of losing

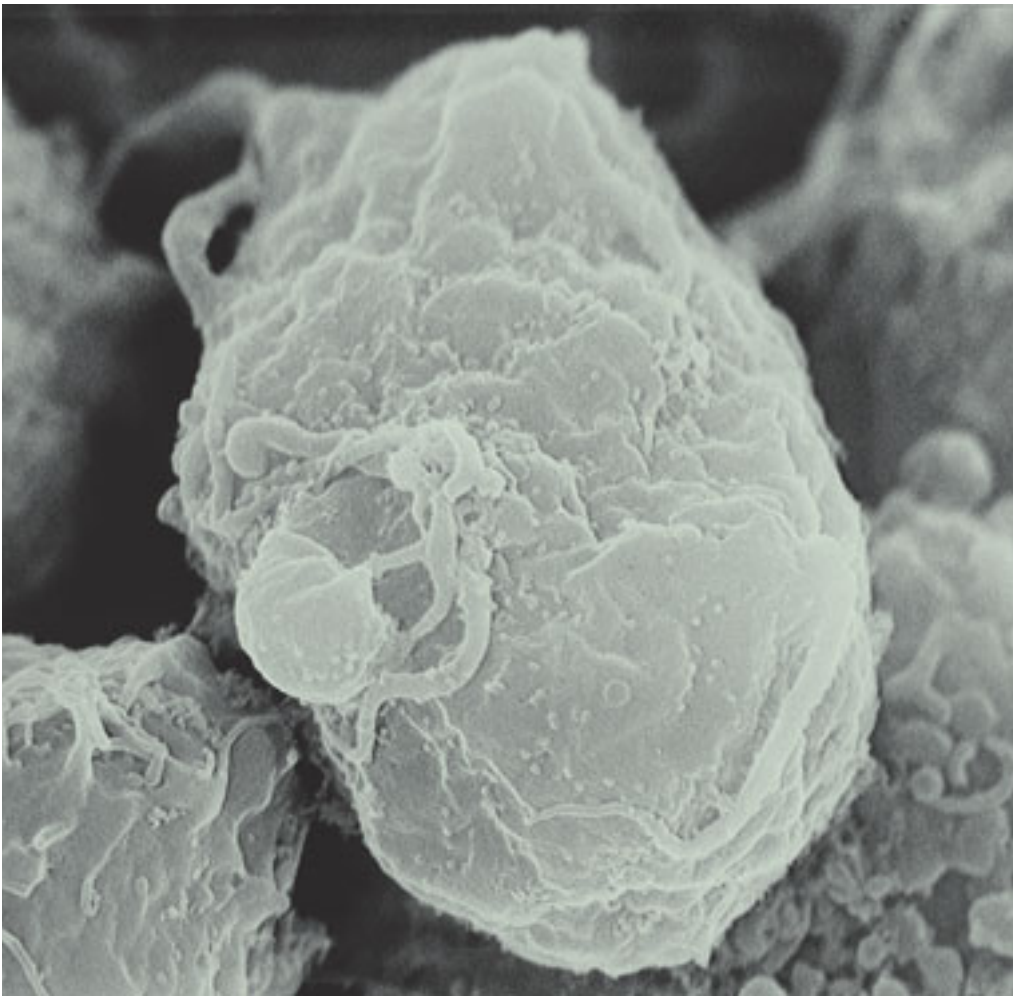
true. The molecular complexities of fat tissue and the difficulties of production and delivery still pose serious obstacles.

And despite all the research advances, obesity is still in many respects uncharted terrain.

"We don't even know yet the location of the genes that very likely make people susceptible to obesity," says Harvard's Flier. "These genes could be active in the brain, or in the fat cells, or in the muscle cells, or really everywhere." Flier believes that the answer most likely will come from large-scale population studies.

Will there ever be a "cure" for obesity? "It's really too early to say," says Lodish. "I doubt a single molecule will ever do the trick. But one might help reduce the problem, especially in the early stages."

In the meantime, here's his prescription: "Diet and exercise."



BEATING TH

Making vaccines against today's epidemic infectious killers is slow,

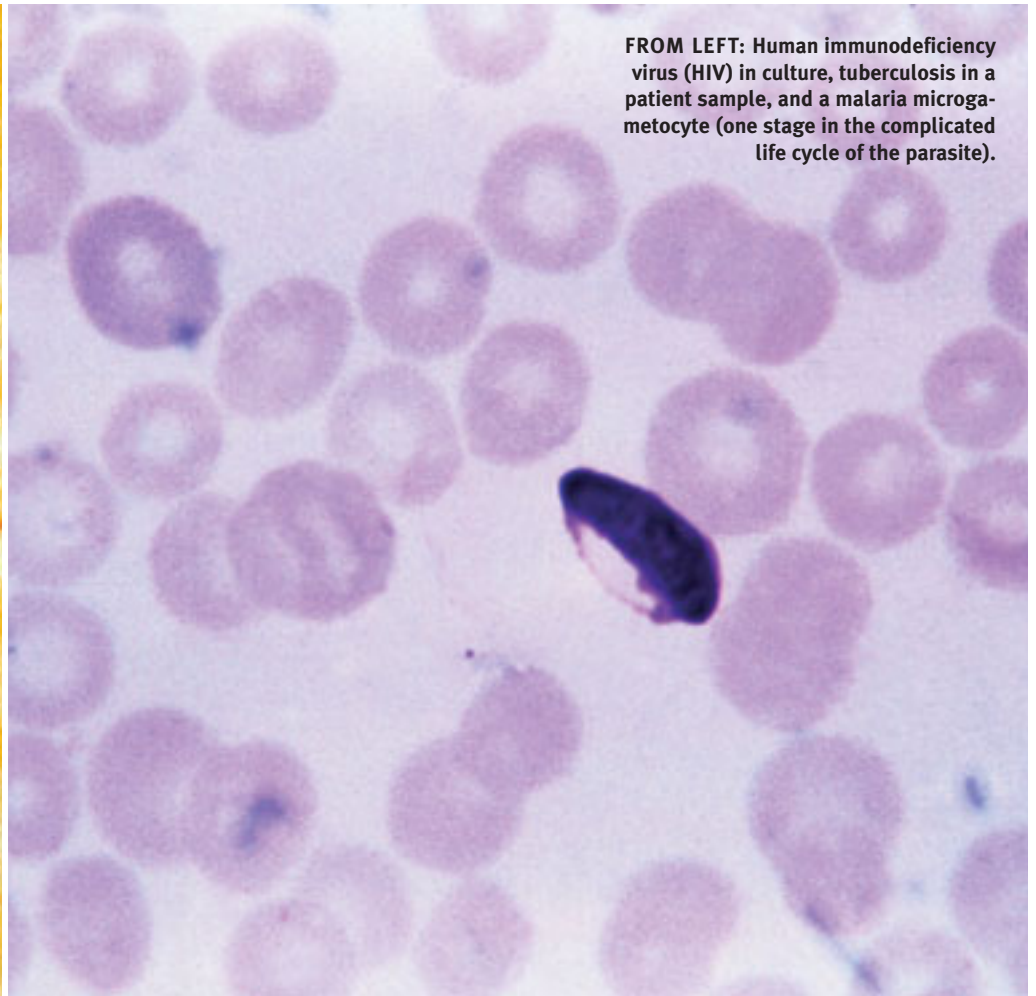
In 1984, scientists announced that the frightening new disease called AIDS had been traced to a previously unknown retrovirus called the human immunodeficiency virus (HIV). “We hope to have a vaccine ready for testing in about two years,” declared U.S. health officials. “Yet another terrible disease is about to yield to patience, persistence and outright genius.”

Two decades later, AIDS has killed over 20 million people worldwide and no proven vaccine is in sight. Aids-Vax, the first (and so far, only) experimental HIV vaccine to enter large-scale Phase 3 testing, was found all but worthless in 2003. While at least two dozen vaccines are in clinical trials and there's restrained optimism that the hurdles will be overcome, some skeptics doubt a safe and effective AIDS vaccine will ever be made.

Progress in making vaccines for the other epidemic scourges of the developing world has been equally slow and frustrating. Together with AIDS, tuberculosis and malaria make up the “Big Three” infections for which vaccines are most urgently sought. Together, these global diseases take an estimated 5.7 million lives every year—a body count that every two weeks equals the death toll of the Asian tsunami. Despite decades of research and trials, vaccines for the Big Three are either inadequate or don't exist.

And at any time, a new threat could appear. Epidemiologists are keeping a wary eye on the massive epidemic of bird flu in Asia that, given the right mutation, could jump to humans. U.S. officials say it would take six to eight months to create a variant of existing influenza vaccine to combat what some fear could be a global flu pandemic.

CDC/C. GOLDSMITH, P. FEORINO, AND E. L. PALMER



FROM LEFT: Human immunodeficiency virus (HIV) in culture, tuberculosis in a patient sample, and a malaria microgametocyte (one stage in the complicated life cycle of the parasite).

THE BIG THREE

frustrating and overwhelmingly important | BY RICHARD SALTUS

Even keeping production levels adequate for the standard flu vaccine is a worry, after the shortages of last year.

Many factors slow the vaccine race: uncertain profitability, companies' fears of liability, ups and downs in research funding, and the staggering costs of large clinical trials.

"All the easy vaccines have been made," says Barry Bloom, a vaccine specialist and dean of the Harvard School of Public Health. "Scientists have been looking for a general formula—if it worked for flu, it should work for something else." Not so, Bloom says, because each pathogen evolves its own strategy to reproduce.

Motivation, however, is strong, given the historic benefits of prevention. In the U.S. and other Western countries, the vaccines developed mainly in the past century represent one of the greatest medical successes in history and one of

the most cost-effective tools in public health. Millions of lives are owed to vaccines against illnesses such as influenza, typhoid, diphtheria, tetanus, smallpox, and polio.

But as they try to extend vaccine development to the major infectious killers rampant today, scientists are contending with organisms that are more complex and deceptive. They are matching wits with organisms that are adept at confusing and evading the antibodies and defensive cells of the body, or even—in the case of the AIDS virus—preemptively attacking the defenses themselves.

"With HIV, you can't get rid of it—it kills your immune system," says Richard Young, a Whitehead Member who uses strategies involving the entire genome of organisms to study disease. "If your immune system can't deal with the bug, there's a real question whether you can ever make a

vaccine against it.” Previously enthusiastic about a novel vaccine strategy for HIV, Young says he’s now considering other approaches to improving immune defenses, using oral drugs.

A CONTINUAL CHALLENGE

The current disease culprits, unlike the hit-and-run agents that cause childhood illnesses, lodge themselves in the body and remain a threat for decades. That means vaccines need to spark immunity that has unprecedented staying power.

The human immune system, an intricate network of specialized white blood cells that congregate in lymph nodes, has two main divisions. One is staffed by B-cells that make antibodies—proteins that come in millions of different configurations and can recognize, bind to and neutralize an invading microorganism by homing in on a distinctive protein, or antigen. The other immune division comprises a variety of T-cells that attack foreign organisms and kill infected host cells.

When the immune system successfully wards off an

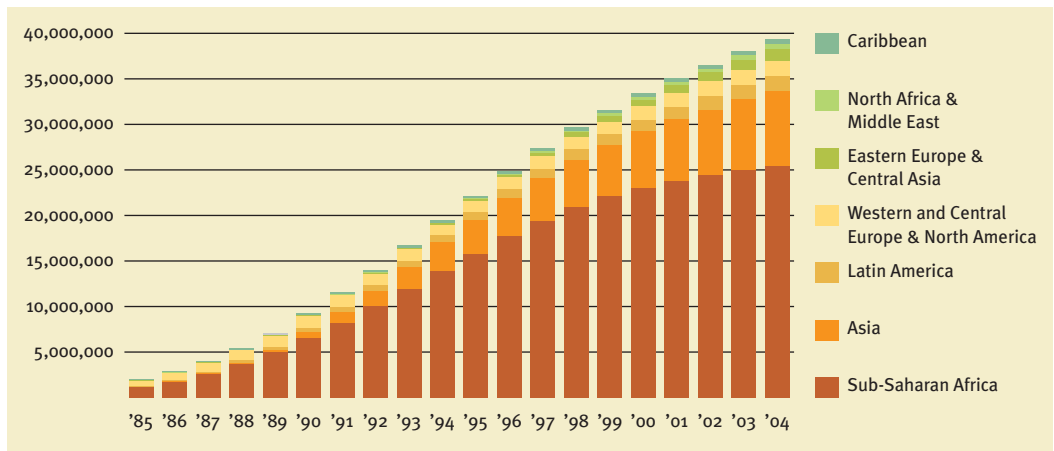


“You have to go where the research takes you, and you get little victories that keep you going.”

—Jerald Sadoff

infectious disease or responds to a manmade vaccine, it creates a standing army of defenders that “remember” the foreign antigen. The next time it detects the antigen, this standing force of antibodies can strike back rapidly. But this memory effect doesn’t always last, and one of the shortfalls of many current experimental vaccines is their failure to provide long-term protection.

Vaccines contain antigens designed to elicit a protective immune response, but do it without causing the disease. Some antigens are weakened disease organisms—bacteria or viruses similar enough to the pathogen to spark immunity but less harmful. Most famously, the smallpox vaccine Edward Jenner created two centuries ago contained the milder cowpox virus. More recently, as with HIV vaccine research, scientists have developed vaccines that contain only a small piece of the virus, such as a protein on the surface of the virus that can stimulate an immune response.



THE HIV/AIDS PANDEMIC

“Effectively tackling HIV/AIDS is the world’s most urgent public health challenge,” says the director general of the World Health Organization. Here are the numbers of adults infected worldwide, from 1980 to 2003. The annual death toll now far exceeds two million.

When they’ve tried this strategy in AIDS vaccines, however, the responses in animal testing have been disappointing.

ANOTHER AIDS ATTACK

Despite the setbacks in clinical trials, the AIDS vaccine saga continues. In January 2005, the National Institutes of Health’s HIV Vaccine Trials Network in collaboration with Merck announced a large-scale Phase 2 clinical trial of a vaccine aimed at provoking cellular immunity.

With little success in eliciting neutralizing antibodies against HIV, Merck and others are turning to T-cell immunity as the best target. Researchers hope to enroll 1,500 volunteers who are HIV-negative but at risk of becoming infected to receive the vaccine. The Merck product uses a weakened cold virus called Adeno 5 to carry three synthetic HIV genes into the body in hopes of spurring T-cells to attack HIV-infected cells and lowering amounts of virus in volunteers who do develop infections.

The Bill and Melinda Gates Foundation currently coordinates the newly created Global HIV/AIDS Vaccine Enterprise, an umbrella for a number of independent agencies and organizations. Last January the enterprise issued a strategic plan aimed at “new opportunities” that include a database of all the clinical-trial data, a large field of candidate vaccines, and improved animal models for testing.

TAMING TUBERCULOSIS

The tuberculosis (TB) bacterium is far more widespread than AIDS, now infecting one-third of the world’s people. Although only 10% of them ever develop clinical disease, TB kills two million of them a year.

The existing TB vaccine, Bacille Calmette-Guérin or BCG, dates to the early 1900s. But its efficacy, as measured by clinical trials, varies from as high as 80% protection in the United Kingdom to almost none in India.

Designing a new vaccine is quicker if scientists can identify some biological marker in people or animals that would

quickly reveal whether the vaccination was working. But no such “correlate of immunity” has been found for TB, even after years of searching for distinctive differences in the immune systems of infected people who remain well compared with those who develop TB.

Neutralizing antibodies, the standing army raised by the infection, haven’t been detected in infected but healthy people, says Jerald Sadoff, president and CEO of the Aeras Global TB Vaccine Foundation in Bethesda, Maryland. So most experimental vaccines currently aim at stirring a response in the other arm of defenders, the T-cells.

Aeras was launched in 1997 with \$83 million from the Gates Foundation, and has provided a huge financial shot in the arm to development of TB vaccines. Aeras has undertaken a small human safety trial of an experimental vaccine, using an improved “recombinant” version of BCG. Eventually, Aeras plans to test a “prime-boost” strategy, giving one vaccine to “prime” the immune system, followed by another vaccine to create longer-lasting protection. “We feel that priming with BCG somehow induces a memory response, and with a boost you get long-lived effector and memory cells,” says Sadoff.

At least two other experimental vaccines are in early trials. One contains two proteins from the TB bacterium that strongly stimulate the immune system. NIH is sponsoring the trial, run by Corixa and GlaxoSmithKline. Another “subunit” vaccine containing an immunogenic TB protein was found safe in a small trial in 2004. Devised by an Oxford University team, the vaccine would be given in tandem with BCG as an “ally vaccine.”

MALARIA’S GENETIC NIGHTMARE

For sheer elusiveness, it’s hard to outstrip the mosquito-borne parasite that causes malaria, *Plasmodium falciparum*. Malaria causes enormous suffering and death in sub-Saharan Africa. A million people die a year, 700,000 of them children. Death rates are higher now than in the past.

The malaria-carrying mosquitoes inject tiny reproductive particles called sporozoites into the victim’s bloodstream. They first enter the liver, expand their forces, and then infect blood cells, killing them and causing life-threatening fevers and anemia. The malaria parasite contains 6,000 genes, and many proteins are expressed at each stage of the life cycle. Since up to five different strains may infect an individual, the parasite can daze and confuse the immune system by displaying as many as 1,000 different antigens.

It may require a combination of vaccines to protect people against malaria, including some that would not protect an individual against infection but might keep the parasite from spreading to others. One piece of good news came in 2004 when a vaccine developed by GlaxoSmithKline and tested in Mozambique protected children from the disease at a rate of 30%, and prevented it from becoming life-threatening 58% of the time. Tests of that vaccine and 14 other experimental products are funded by the Malaria Vaccine Initiative with Gates Foundation backing.

Overall, research on the Big Three is making important progress, says Aeras’s Sadoff, and he’s excited at the way what’s learned about individual diseases is converging. “You have to go where the research takes you, and you get little victories that keep you going.”

Reversing the research

Historically, those developing vaccines had to be prepared to spend years or even decades in the process—coaxing the disease-causing organism to grow in the laboratory, isolating its many antigens, determining which are most likely to induce immunity in a host, and conducting lengthy clinical trials of several candidate vaccines. It’s a long road, especially if, as sometimes happens, it proves impossible to grow the pathogen in culture at all.

But the advent of DNA sequencing and genomic technology has brought new tools to the field of vaccines, making antigen discovery faster and more systematic, and creating shortcuts that reduce the need for hands-on work in the laboratory.

In one method, dubbed “reverse vaccinology,” scientists turn the process around. They start by scanning the organism’s DNA blueprints and determin-

ing which genes make useful antigens. This step is aided by algorithms that predict the structure of the proteins the cell will make from the DNA blueprints.

“In reverse vaccinology, you don’t even care about the pathogen,” explains Reno Rappuoli, chief scientific officer of Chiron in Emeryville, Calif. “You never see the pathogen. You just go to the computer to generate your predictions.”

Researchers have decoded the genomes of more than 140 bacteria and 1,600 viruses. Rappuoli says the set of sequences for each organism represents a complete “virtual catalogue” of all its antigens. From these, he says, “it is possible to select the molecules that are likely more effective.” Generally, these are proteins embedded in the surface of the pathogen’s cells and which are first detected by the immune system of the infected host, prompting a defense response.

Finally, the hunt moves to the laboratory, where *E. coli* bacterial factories equipped with the antigens’ genetic blueprints turn out quantities of pure antigen. The different types of antigen are harvested and tested in animals for their ability to provoke a protective immune response, and the most successful become candidates for human clinical trials.

Chiron has used the reverse method to create a vaccine against a particular strain of the bacterium *Neisseria meningitidis*, the cause of a form of meningitis that has defied vaccine developers for decades. The company also exploits genomic tools in the development of vaccines for hepatitis, HIV, *H. pylori* (the cause of stomach ulcers) and cancer, says Rappuoli.

Genomics also has aided TB vaccine research, says Jerald Sadoff, president and CEO of the Aeras Global TB Vaccine Foundation. “It has provided us with innumerable new antigens that can become candidates for vaccines,” he explains.

BELOW: A member of Whitehead's Scientific Advisory Board, Elaine Fuchs helped to found the field of skin biology.

OPPOSITE: The Fuchs lab has identified key proteins involved in organizing skin cells (blue) into three-dimensional tissues.



ELAINE FUCHS always has had a sense of adventure. As a senior studying chemistry at the University of Illinois, Urbana-Champaign in the early '70s, she hoped to go to Chile with the Peace Corps after graduation. When she ended up assigned to Uganda, Fuchs decided Idi Amin's oppressive regime would be too much for her. She opted instead for graduate school in biochemistry—with just a single biology course on her undergraduate resume.

"Biology was always a bit problematic for me," she recalls. "There were always too many variables to solve an equation. And yet, I liked the idea of having my science more closely related to some aspect of medical importance."

Three decades later, Fuchs has learned not only to live with uncertainty but to thrive upon it, having helped to found the field of skin biology and remaining a leader within the field. "She is invariably at the very forefront of the field—often before anyone else realizes it is the forefront—asking the most insightful questions in the most powerful way," says Paul Khavari, a dermatologist and skin biologist at Stanford University.

Last year Fuchs continued that streak. She published papers in *Science* and *Cell* that described the first methods for isolating, purifying and characterizing epithelial stem cells directly from their niche in the skin.

LEARNING HOW SKIN RENEWS

You can trace the stem cell papers back to the beginnings of Fuchs's career. After finishing her PhD at Princeton University, she chose a postdoctoral position with Howard Green at MIT, studying growth and differentiation with a new system developed in the Green lab for culturing epidermal cells.

"I didn't like the idea that virtually all of the cell culture systems—this

Under

ELAINE FUCHS PIONEERS OUR UNDERSTANDING OF SKIN BIOLOGY—AND ADULT STEM CELLS

is ironically true to this date—were immortalized cells, cells that are somehow genetically transformed so that they divide in culture,” says Fuchs. Green’s system was one of the few exceptions that maintained normal, non-transformed cells.

The reason? The skin’s enormous natural reservoir of stem cells.

The human skin provides an incredible protective barrier for the body. It is constantly rejuvenated in order to provide that barrier. Every two weeks the skin generates a brand-new epithelium, or outer covering. To accomplish that self-renewal, the skin contains uncounted numbers of stem cells.

“You can put those cells into culture, and those cells will divide and maintain their properties,” says Fuchs. “That’s really why I got into this system.”

“When I began in the field, we knew virtually nothing about the skin epithelium,” she says. Most of Howard Green’s lab was studying how the cells divide and behave. Fuchs wanted to understand whether there were cells that could become all of the types of cells seen in skin: the epidermis, fat-secreting sebaceous glands and hair follicles.

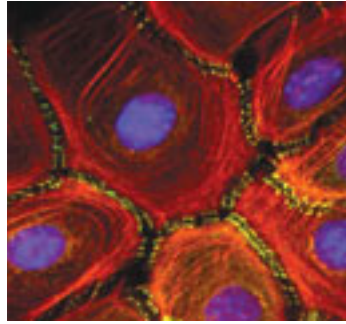
After her postdoc, Fuchs established her own lab at the University of Chicago and effectively launched the molecular genetic study of skin. “It was essentially starting a field from scratch,” she says. “It takes a long time to set the foundation before you can really start to ask what I now find are the most exciting questions.”

Fuchs answered a number of key questions about the growth, development, and structure of skin and hair, including the roles of several genes in human genetic skin diseases. Her lab pioneered the use of reverse genetics in the study of skin disease, a process in which researchers disable a gene in a mouse and observe the results.

PINPOINTING STEM CELLS

Fuchs moved from Chicago to New York’s Rockefeller University in 2002. In January 2004, she and co-workers published a paper in *Science* outlining a method for labeling and purifying cells from the skin’s stem cell niche.

Her group genetically engineered mice to express a marker



models that she’s created will allow us to finally ask a lot of the basic questions in skin biology.” Stanford’s Khavari

believes the methods will also help biologists study tissue regeneration, gene therapy and cancer.

Fuchs now plans to tackle both basic and applied problems, starting with studies about what makes skin stem cells self-renew,

“It takes a long time to set the foundation before you can really start to ask what I now find are the most exciting questions.”

called green fluorescent protein, or GFP, in skin cells. After allowing the mice to grow and make GFP for four weeks, the scientists turned off the GFP gene. Over time, ordinary skin cells died off, losing their glow. The stem cells kept shining.

Despite experiments to characterize the cells and to demonstrate their capability to form hair follicles and new epithelium, Fuchs still felt they had not been definitively identified as stem cells. Then in September a second paper appeared in *Cell*, which rigorously showed that the labeled cells displayed the two defining characteristics of stem cells, self-renewal and the ability to form multiple kinds of differentiated cells. Depending upon the biochemical signals they receive, these cells can form epidermis, sebaceous glands, or hair follicles.

“It’s the first time that our field has had a way to really track the fate of stem cells in vivo,” says Angela Christiano, who studies the biology of hair loss at Columbia University. “These

what makes them decide to choose one lineage over another lineage, and to what stimuli they respond.

Ultimately, Fuchs says, she wants to discover whether biologists can learn to manipulate the cells to do something they normally wouldn’t do, say, create a corneal epithelial cell. If so, the cells might aid treatments not only for burn operations or hair replacement, but for blindness or chronic ulcers, for instance.

“She’s a pioneer when it comes to applying new technologies to skin-related questions,” says Christiano. “She picks a tough, seminal question and then puts together, using the cutting-edge technology, a series of experiments that really get at the mechanism of why something happens.”

“Outstanding scientists can be visionary thinkers who see new advances before they materialize, or they can be brilliant experimentalists who definitively move the field forward,” adds Khavari. “Elaine is both.”

COURTESY OF ELAINE FUCHS

your skin

BY ERIKA JONIETZ

AS MEDICAL DIAGNOSES RACE FURTHER AHEAD OF PREVENTION AND TREATMENT, PATIENTS AND THEIR FAMILIES WONDER WHEN TO TEST **BY ERIC BENDER**

The price of prediction

Do you want to know the odds that you or your child will get a life-threatening incurable disease? Increasingly, that choice is yours.

Today, there is a diverse and quickly growing array of medical diagnostic tests, with astonishing improvements in everything from antibody detection to genetic testing for cancer risks to detailed images of what your brain is doing right now. But the information these tests churn out can lead to potentially disturbing decisions—and make us wonder exactly what we want to know about our medical futures.

TYPE 1 DILEMMA

Take type 1 diabetes, which strikes about one in 300 children in the United States. Also known as juvenile diabetes, the autoimmune disease wipes out the cells in the pancreas that make insulin. Your body needs the hormone to get glucose into your cells. Without insulin, you die.

Fortunately, type 1 patients benefit from a series of remarkable scientific achievements. Slick equipment for testing blood glucose levels and delivering insulin helps type 1 patients live fairly normal lives and minimizes the chances of complications such as blindness and kidney disease. But the disease is difficult to manage, and so is the fear that the patient's brother or sister will develop the disorder as well.

Enter Type 1 Diabetes TrialNet, launched by the National Institutes of Health in 2002. The massive project is testing relatives of those with the disease, starting with a check for several autoimmune antibodies that help to predict the disease.

Most get a clean bill of health. Those who don't move on to genetic screening for a handful of genes highly implicated in the disease. They're also re-tested at regular intervals, to avoid the need to be hospitalized if the disease strikes. Some will be eligible for TrialNet experimental treatments to delay, prevent, or minimize it.

TrialNet "opens the way to potentially develop new

treatments and to prevent the disease," emphasizes Judith Fradkin, who oversees the program as director of the Division of Diabetes, Endocrinology and Metabolic Diseases within the National Institute of Diabetes and Digestive and Kidney Diseases.

While TrialNet offers a safety net of sorts to participants, the testing isn't foolproof. And what do you do, say, if you learn there's a 25% chance that your child will develop the condition within five years?

"We can predict type 1 diabetes in man, we can prevent it in animals, but we don't know how to safely and effectively prevent it in man," says George Eisenbarth, executive director of the Barbara Davis Center for Childhood Diabetes at the University of Colorado Health Sciences Center in Denver.

The decision to test "is still a balance between anxiety and information," acknowledges Eisenbarth, who pioneered antibody testing for the condition. "I lean on the side of giving the information."

"It's a dilemma," says Jennifer Barker, a medical doctor and researcher who works with Eisenbarth at the Barbara Davis Center. "If results are negative, you give a sigh of relief. If they're positive, you're waiting for that ball to drop, and you have the stress of knowing something that you can't do something about."



GENETICS, UP CLOSE AND PERSONAL

Genetic testing, which in the future will affect all of us, is the poster child for the delivery of useful, but incomplete, information.

Pre-natal genetic testing has become commonplace for high-risk pregnancies, providing reliable predictions of Down syndrome and selected other conditions. Along with that comes agonizing decisions for some expectant parents. Down the road, parents may get genetic analyses whether they ask for them or not, as the U.S. medical system debates a controversial proposal to test all newborns for 29 conditions.

Other genetic analyses also are booming. Your DNA can be clinically examined for your risk of hundreds of diseases. The list is growing. Tests for cardiac and psychiatric conditions will arrive within five years, predicts Anne Greb, president of the American Board of Genetic Counseling and director of the Genetic Counseling graduate program at Wayne State University School of Medicine in Detroit.

Before any genetic testing is done, its usefulness must be assessed, Greb emphasizes. "A big part of what we do is risk assessment: Is genetic testing worth it for this family? Most of the time it's not." Counselors also promote ethical standards. For example, children generally are not tested

unless knowing their genetic status is important for medical management.

DNA Direct, an online firm that offers direct genetic testing, covers only conditions that are actionable, says clinical director Elissa Levin. "We offer scientifically valid tests such as hereditary blood clotting and iron overload, so that if you know your risk to develop a condition is increased, you can take definitive actions to reduce that risk through medical management, surveillance, lifestyle changes and knowing early warning signs," she says.

A LITTLE KNOWLEDGE

If patients go ahead with the testing, that's when the truly tricky part begins, as genetic counselors help them to understand the results.

For a small minority of diseases, results are fairly clear. "We found that people who have a certain gene regulator are almost certain to develop type 2 diabetes by the time they're an adult," says Whitehead Member Rick Young. "Here the cost-benefit of testing might be adequate, because patients can modify their diet or take a therapeutic to address the disease."

That's an exception, though, since most diseases are not as well understood and involve multiple genetic factors and environmental stresses. "There are very few tests that can tell you definitely yes or no about something," Greb says.

And as Levin points out, risk is very difficult to convey. "Some people just want to know, is this a yes or a no? But genetics doesn't work that way. The role of the genetic counselor is to help interpret risk and educate people to help them make informed decisions."

The information that goes into such decisions is evol-

"There are very few tests that can tell you definitely yes or no about something." —Anne Greb

ing rapidly, along with our understanding of the genetic components of disease. One example is the BRCA1 gene implicated in breast and ovarian cancer. Scientists once thought that women with an altered version of the gene had an 85% chance of developing cancer, odds that prompted many women to have their breasts and ovaries removed as preventive measures. Now, researchers estimate the risk as closer to 50%.

As scientific understanding improves, so will the tools for genetic testing, eventually perhaps including a patient's entire genome. "Microarray-based testing should be very reliable, and very expensive," Young says.

He expects that within five years, genetic testing for conditions that physicians can act upon prior to onset will be much more routine. "Ten years from now, this is probably the way that we do a lot of preventive medicine," he adds.

By then, families participating in TrialNet hope, type 1 diabetes will be preventable and curable. And their dilemma will be over.



Global warming for stem cells

Research policies vary widely, but the climate's getting a little friendlier

SINCE 2001, when the Bush administration prohibited federal funding for scientists working with newly created human embryonic stem cell (HESC) lines, the U.S. map has turned into a crazy quilt of conflicting state regulations. Overseas you'll find a similar mish-mash, ranging from the outright prohibitions in Italy to the liberal guidelines of the United Kingdom.

The trend is clear, however. As in Asia, where opposition to HESC research has never been very significant, in Europe guidelines have steadily loosened, and public opinion seems to be moving in the same direction. In November, for example, 66% of the Swiss electorate voted to allow the use of surplus embryos in HESC research.

We're seeing a "rapid liberalization of human embryonic stem cell research policy occurring in many nations," says LeRoy Walters, Joseph P. Kennedy Professor of Christian Ethics at Georgetown University, who recently reported on HESC policies to the National Academy of Sciences.

Probably the most common position is support for HESC research except for somatic cell nuclear transfer (combining a nucleus with an egg from another organism, also known as therapeutic cloning). Over a dozen European nations plus Australia, Canada, Iran, and Taiwan take that position. Japan,

"We're seeing a rapid liberalization of human embryonic stem cell research policy occurring in many nations." —LeRoy Walters

Singapore, and Sweden also accept nuclear transfer. Belgium, China, Finland, India, Israel, South Korea, and the U.K. also accept HESC lines derived from in vitro fertilization.

Since 2002, says Walters, 13 nations have moved to less restrictive policies.

As in the U.S. where a mid-2004 Harris Interactive Poll showed that 73% of the public supported HESC research, government policies don't always go hand in hand with popular opinion. For example Finland has a liberal policy, yet a November 2003 EOS Gallup Europe survey of over 15,000 Europeans indicated that only 43% of Finns supported HESC research. On the other hand, 65% of Italians surveyed were supportive, despite their government's opposition.

Overall, men were slightly more favorable to HESC research than women. The big difference was in age, with younger people much more likely to show support. —Eric S. Brown

Public offerings

Funders increasingly ask researchers to share data and software tools

SHOULD FEDERALLY FUNDED investigators make their papers freely available, after the papers are published in journals such as *Science* and *Nature*? Early this year the debate on that question was capped by a National Institutes of Health policy that requests but does not require that NIH-funded scientists submit their final, peer-reviewed papers to the National Library of Medicine's PubMed Central. A year after publication, the papers would be publicly available to anyone on PubMed.

But while that debate simmers, a related trend is quietly gaining steam: requiring scientists to share their research data and custom software tools.

"An awful lot of research data is ending up in bit heaven somewhere," rather than helping other scientists with their studies, says Mark Ellisman, director of the Biomedical Informatics Research Network coordinating center in San Diego. That's simply not the best scientific practice. "Funding agencies don't want to fund things that are just used by one person," points out Fran Lewitter, director of Whitehead's Bioinformatics and Research Computing (BaRC) group.

Whitehead is among the institutions leading the effort to release research data publicly. The Institute plans to roll out a number of resources for public use this year, including the Whitehead Array Database Exchange, which will be a one-stop shop for publicly available data.

Lewitter says that the sharing of data analysis software is also on the upswing, but it's trickier. "It takes a little extra effort to build software you can actually put out there," with suitable documentation and support, she says. Whitehead already offers a few software tools, among them the siRNA Selection Program, a popular Web-based tool that speeds work in studies employing RNA interference. —Jennifer Tomase



Fast science speeds to the rescue

CDCP head calls for rapid response to new health threats

WHETHER THE THREAT comes from birds, pigs, monkeys or bio-warfare laboratories, the chances are high for an outbreak of disease far deadlier than the Severe Acute Respiratory Syndrome (SARS) event of 2003.

One prominent example is the threat from the HN51 virus, a subtype of the avian influenza virus now percolating in Southeast Asia. This could infect up to 30% of humanity and kill up to seven million, estimate World Health Organization officials.

Fortunately, public health officials are gearing up to prepare for the newly emerging threats.

Julie Gerberding, director of the Centers for Disease Control and Prevention, is hammering home the need for improved communications networks and collaborative "fast science" to more quickly control rapidly emerging infectious diseases.

"Science has to be faster...not just in the publication and dissemination of results, but in the production of information just in time," Gerberding told a meeting of the Association of American Medical Colleges last fall. "The days of dotting every 'i' and crossing every 't' before research findings are released...are gone in the context of an emerging health threat."

The rapid response to SARS showed how "fast science" could be made to work on a global scale, says Charles Safran, chairman of the board of the American Medical Informatics Association. Almost a dozen laboratories worldwide worked together to identify, isolate and work out the viral genome for SARS before the outbreak got out of control.

"You have unprecedented international cooperation among scientific labs now," says Safran. The greater need is to improve early-detection systems to give researchers a head start. Many of these problems are being addressed by the CDC's new Public Health Information Network.

New software is needed, says Safran, for spotting early signs of an epidemic by tracking hospital records and pharmaceutical sales. The CDC's new BioSense program will integrate early-warning indicators and provide tools to identify trends.

Additionally, health professionals must be trained and encouraged to act quickly and decisively. "We've got to be training students to profoundly embrace the concept of connectivity," Dr. Gerberding told the AAMC. "And we're going to have to do everything we do much faster." —Eric S. Brown

Malaysian nurses screen motorists from Singapore for SARS at an immigration checkpoint.

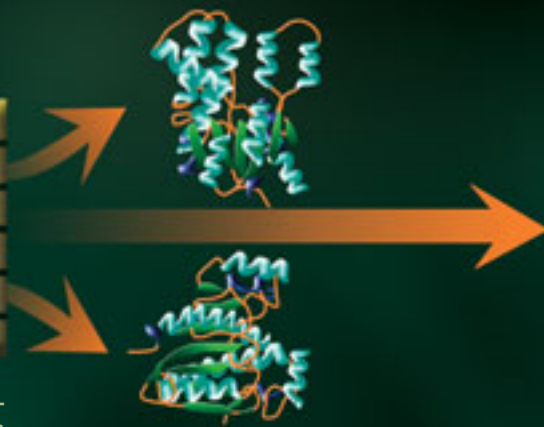
WADE
Whitehead Array Data Exchange

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First, you try “homology modeling”, accessing a database of known protein structures and seeing if the protein sequence in question resembles anything in this database. If so, you can use that structure as a template to guide your work. Sadly, this option doesn’t always find a good match.

Another option is “threading”. Here, a computer program examines the shape of about 15,000 proteins in the structure database that have been imaged in 3D. It also analyzes the amino acid sequence of your protein and predicts the probability that your particular sequence can form the shape of each structure in the database.

Visualizing proteins

To understand how proteins interact with each other, you need a three-dimensional model of each protein. Here’s how you build the model.

A gene is a lot like a line of computer code. To visualize it, all you need to know is the linear string of chemicals that make up its sequence on a DNA molecule.

Not so with a protein. While proteins are also made up of strings of chemicals, simply knowing the order of those chemicals (amino acids in this case) doesn’t tell you much about how the protein is structured.

But knowing the shape of a protein is an important tool for understanding its function and how it interacts with other proteins. And this is another area where computer technology has revolutionized the life sciences, because a protein is so tiny that even the best microscopes can’t reveal all of the molecule’s intricacies.

When scientists speak of a protein’s shape, they’re referring to something that looks like a

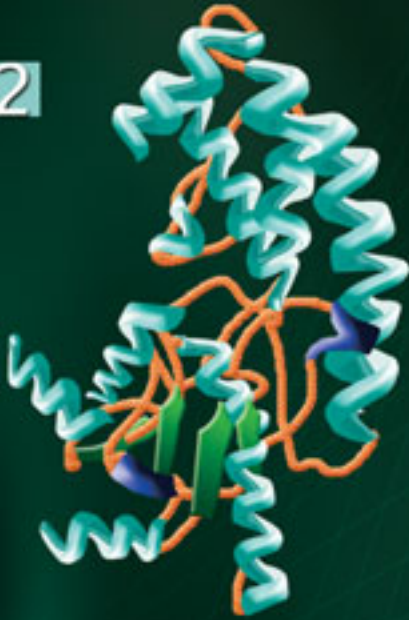


Finally, you mold a “skin” around this skeleton to represent the protein’s shape. You then convert all of this information into an image. Soon, a clear, working model of the protein emerges, one that researchers can take back to the lab.



At this point you have a working model of a protein, one that tells you about every single atom of every amino acid. Now the program connects the dots by joining all the atoms together to produce the protein’s “skeleton”.

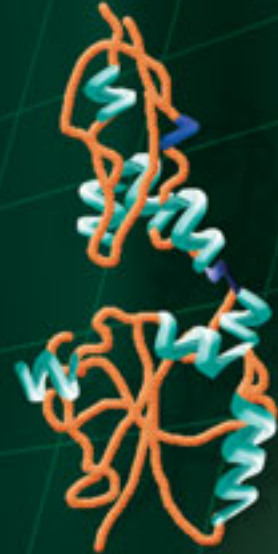
2



Essentially, the computer performs a statistical comparison and eventually picks what it considers to be the most likely shape that your protein may adopt. This is a “template hit”. You now have a rough draft of the protein’s shape. This template now becomes the scaffold upon which to build your protein’s model.



3



Now you take your sequence and wrap it around the protein scaffold, almost like papier mâché. This will give you a basic 3D shape.



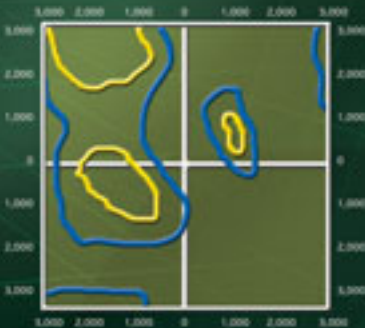
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Next, refine this 3D shape, starting at one end of the protein and working your way to the other, seeking the most feasible conformation. The computer performs an energy calculation, deciding, for example, how closely two portions of the protein can approach each other.



5



As you run the structure through this program, it makes graphical depictions to aid you in correcting errors manually. Eventually you develop the most likely model.



The super bug battle

How bad is antibiotic resistance, and why are drug companies doing so little about it?

ROUGHLY 70% of the bacteria responsible for hospital-based infections are resistant to at least one of the drugs most commonly used to treat infections, says the Food and Drug Administration. Even worse, some bacteria seem to outsmart *all* known drugs. **B. Joseph Guglielmo**, professor of clinical pharmacy at the University of California/San Francisco's School of Pharmacy, and his colleagues look for solutions. He gave associate editor David Cameron a quick update on the antibiotic challenge.

What's the core of the problem?

There are two types of bacteria: gram-stain positive, and gram-stain negative. (This refers to a staining test that's been used for many decades in hospitals all over.) We've seen a large increase in resistance in both gram positive and gram negative bacteria. But over the last ten years the real issue is gram negative. An example of this is *E. coli*, the most common bacteria to affect patients in hospitals. Gram negatives are responsible for most hospital-borne infections.

We also have resistance problems with gram positives, like staph aureus and streptococcus, but there is a pipeline from pharma that addresses this. There's also a drug pipeline for anti-fungals and anti-virals. But nothing for gram negatives. In fact, we now have patients in hospitals with bacterial infections and no antibiotic for it.

What do physicians do?

They use combinations of antibiotics and pray for synergy. They also dust off some older, far more toxic antibiotics and hope they work.

Why isn't big pharma all over this?

For a while there weren't many drugs that worked for gram positives, so companies went after that. But they're now having second thoughts. The return on investment for antimicrobials is not very good. Companies would rather invest in something you'll take the rest of your life than something you take for only seven days.

How long has resistance been a problem?

Take Cipro, the anthrax drug. It's been great for treating gram negative infections. When it first started being used, around 1990, *E. coli* resistance was unheard of. Today,

the rate of *E. coli* resistance to Cipro is 30%. In the past the medical establishment has thought, well, pharma's gonna bail us out. That's not the case.

Is over-prescription really to blame?

Absolutely. We created this situation. Most sore throats and sinus infections don't benefit from antibiotics because they're viral. Nevertheless, the public often demands it, and health care workers give in.

So if someone gets pneumonia, they're often treated with a broad spectrum of super antibiotics just to make sure the resistant ones aren't missed. If that patient languishes in the hospital and gets a new infection, you need an even stronger antibiotic. You go from a pistol to a rifle to a cannon to a missile.

But bacteria are smart. They learn how to evolve.

What's the solution?

First, prescribers *and* patients need to be more aware of the perils of over-prescribing antibiotics.

Second, hospitals must aggressively pursue antimicrobial management programs that work closely with infection control programs, avoiding sloppy behavior that exposes patients to organisms.

But there's also antimicrobial stewardship. I oversee the antimicrobial stewardship program here at UCSF. We have infectious-disease consultants authorized to terminate unnecessary antibiotic use even if it goes against the orders of the primary care physician.

Would that solve the problem or just slow it down?

Who knows? But there's data out there that shows when you had pockets of resistance to a given antibacterial and stopped using it, the resistance pattern reversed itself. It won't happen overnight, but I'm optimistic that we can put a dent in this problem.

Stem cells à la Whitehead

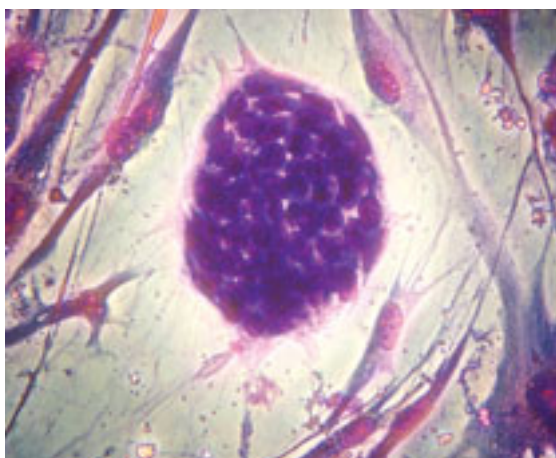
Among all the hopes and political controversies about stem cells, it's easy to forget how little we know about their basic biology. How do embryonic stem cells differentiate into every cell in the body? How can adult stem cells be isolated and grown? Without understanding such basic questions, progress in developing medical applications will be hit or miss. Whitehead researchers have made major advances in this next frontier of biology, and the Institute is now launching its Human Embryonic Stem Cell Facility. For more details, see our On Topic coverage at www.whitehead.mit.edu/news/ontopic/stemcells.html.

Ride the protein roller coaster

Active in nearly everything cells do, proteins are the workhorses of life. These chains of amino acids fold into amazingly complex 3-D shapes, and their interactions with other proteins are what drive biological processes. Whitehead's Bioinformatics and Research Computing group has put together a Quicktime animation that zips you around a sample protein and delves deep into its structure. Put on your bioinformatician hat and check out the animation at www.whitehead.mit.edu/news/academy.

Magic of embryos

Soon after fertilization, a developing embryo must decide just exactly how it will develop. Whitehead Member Hazel Sive investigates how embryos make these early patterning decisions and, in particular, create the pattern for neural development and brain formation. Understanding this process may yield new insights into birth defects, neurological disease, and spinal cord injuries. Sive introduces her field of work in "Form from the Formless: The Awesome Power of the Embryo," at www.whitehead.mit.edu/news/academy.



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In the past few years microarrays have turbocharged gene expression studies. Now researchers in the lab of Whitehead Associate Member David Sabatini are building cell-based arrays, accelerating studies of pathways in living cells. For more information, see page 8.



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