The new age of bioimaging

Digital microscopy and powerful software are turbocharging systems biology

PLUS
How stem cells are wired
A traffic cop for Parkinson’s
Window on Whitehead

Great expectations

“The leaps forward, to the extent they came, were made possible by those who, for so many years, paid for our work yet held back from telling us what to do. They urged us, instead, to go out and indulge our curiosity, to solve the problems that intrigued us, to play little mind games with nature. Maybe you’ll just turn up something interesting, they said, something that in unpredictable ways will lead you a bit closer to the ultimate answer. Go play your games, they said. And we did.”

That’s how Whitehead Member Robert Weinberg summed up his happy dealings with the National Institutes of Health and other funders of biomedical research, in Racing to the Beginning of the Road back in 1996. And that’s the environment in which biological discovery blooms best.

But that environment is under heavy pressure these days, and one of the reasons is public frustration with the rate of medical progress.

NIH head Elias Zerhouni tackled that issue head-on during his Congressional budget testimony this spring, backing the case for the nation’s embattled medical research agency by listing striking advances this country has made in health. Among them, “this year, for the first time in history, the absolute number of cancer deaths in the United States has decreased,” Zerhouni declared.

Health care currently costs the average American about $7,100 a year, while we shell out $95 for NIH.

The rate of medical progress does feel grindingly slow if you know someone, say, slipping into Parkinson’s disease. And no matter how carefully scientists describe their early results in the lab, they are deluged with requests for help from patients and their families. After Susan Lindquist’s lab announced progress in reversing Parkinson’s syndrome in animals (see “Traffic report” on page 14), she received hundreds of desperate emails.

Sometimes medical science is not oversold but overbought.

“Public expectations have been completely redefined by advertising,” comments Duncan Kuhn, an MD and visiting scientist in the lab of Whitehead Member Gerald Fink. “Turn on the TV at night, and every other ad is for a drug. At the same time, people are almost completely ignorant of the process between basic discovery and medical application.”

“People are often insufficiently aware that biomedical research is largely based on discovery,” says Whitehead Fellow Andreas Hochwagen. “You need the right people, the right ideas and the right amount of luck.”

The challenges become clearer as we delve deeper into life’s complexities. “As we drill down in biology, the number of different mechanisms we can identify is equivalent to the number of ideas we have,” as Whitehead Member Richard Young once commented wryly.

But we shouldn’t underestimate the progress that biomedicine does make. “With HIV, we went in relatively few years from nothing to a broad array of therapies,” Kuhn says. “In the early days a lot of people died in front of me, because we had no therapies. Now, I tell patients that they will live to be grumpy old men.”

For $95 a year, that’s an excellent return on investment. —Eric Bender

On the cover

Whitehead postdoctoral fellow Jason Moffat silenced particular genes in human colon cancer cells, fluorescently labeled three components of the cells, and sorted them by specific visual properties. For more information, see “Faces of mitosis” on page 20. Image courtesy of Jason Moffat

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Since many viruses have spent hundreds of thousands of years fine-tuning their abilities to hijack the cellular processes of other organisms, they can tell us a great deal about how our own cells operate.

Investigating one form of the herpes virus, researchers in the lab of Whitehead Member Hidde Ploegh have discovered a key component in the machinery with which cells dispose of misfolded proteins.

“Viruses and other pathogens are simply mirror images of our immune system,” says Ploegh, senior author on the article published in June in *Nature*. “The two have really co-evolved. By studying one, we learn about the other.”

Cells have a very elegant process for disposing of proteins that have mutated or misfolded, a process that involves a cellular organ called the endoplasmic reticulum, or ER.

The ER is a factory of sorts, the site where proteins learn how to assume their requisite shapes. But a lot can go wrong when a protein folds. Moreover, cellular life exposes proteins to lots of wear and tear.

In order to prevent misfolded proteins from accumulating and causing conditions such as Alzheimer’s and Parkinson’s, the ER can dispose of these molecules through a process called dislocation.

In dislocation, the ER marks broken proteins with a chemical tag that flags them for disposal. Once ejected from the ER, a complex called the proteasome captures the flagged protein and shreds it to pieces (see figure above). The protein’s remains are then sent back to the ER where a mechanism called the MHC (major histocompatibility complex) shuttles the fragments up to the cell surface and the immune system examines them for viral traces.

The endoplasmic reticulum (ER) acts as a protein factory. A malformed protein is flagged for disposal, ejected from the ER and shredded by the proteasome. Scraps of the protein are sent back into the ER, where the MHC complex shuttles them to the cell surface and the immune system examines them for viral traces.

Of herpes, can trick the cell into mistaking the MHC for a misfolded protein, which the cell then puts out with the trash. Without the MHC, the cell can’t alert the immune system to a viral presence, and the virus can proliferate unencumbered.

“This virus has spent a long time looking for this pathway’s Achilles heel,” says Ploegh, who is also a professor of biology at MIT. “For that reason, it’s an invaluable resource for probing this dislocation pathway.” In other words, to learn more about how the cell disposes of misfolded proteins under normal conditions, one should closely study how HCMV operates.

The Ploegh lab singled out two proteins, US2 and US11, essential for the herpes virus to bypass immune detection by shuttling MHC into a degradation pathway. Most recently, graduate student Joana Loureiro and colleagues found that a protein called SPP (signal peptide peptidase) cooperates with US2 and is essential for the virus’s ability to disarm the cell.

“We now believe that we’ve stumbled over a previously unknown function for SPP in helping the cell get rid of malformed proteins,” says Loureiro.

Normally, SPP’s job is to break up small proteins called signal peptides which are important for other aspects of immune surveillance—a function not related to dislocation.

“There are many common diseases that are caused by expression of a defective form of a protein, like cystic fibrosis, or accumulation of misfolded proteins, as is thought to be the case for Alzheimer’s,” continues Loureiro. “Any molecule that we can find that contributes to the general process of ER protein disposal is an important discovery.”
Men differ on Y

*Male chromosomes are far more diverse than we thought*

Try tracing your heredity purely on the basis of your genes, and you’ll immediately run into trouble. Chromosomes, after all, come in pairs, one from your mother, and one from your father. Like a deck of cards, they’re shuffled around each time a baby is born. It doesn’t take more than a generation for the genetic trail of evidence to become hopelessly muddled.

Not so with the Y chromosome. The male-exclusive Y is passed on like a baton from father to son, unscathed. If, somewhere down the family tree, a man’s Y differs from one of his ancestors, this departure resulted from a mutation and not from the mixing of gene pools.

Reporting in *Nature Genetics*, researchers in the lab of Whitehead Director David Page showed that large-scale structures of Y chromosomes are far more diverse than previously thought—and significant mutations involving sizable chunks of the chromosome can occur in a single generation.

“We had suspected for some time that the structure of the Y chromosome predisposed it to rearrangement, to gene loss and gene gain, but we didn’t know how common this was,” says Steve Rozen, a senior research scientist and senior author on this paper. The population groups represented in these samplings were vast, including bushmen from South Africa, Melanesians, South Americans of pre-Columbian descent, as well as western Europeans. While differences between groups were sometimes striking, researchers still found significant varieties among men within the same group, such as differences in the number of gene repetitions, chunks of the chromosome flipped around, and variations in the ultimate size of each individual Y. And from an evolutionary perspective, these variations were all quite recent.

Many of these variations involve sperm-producing genes. While the researchers don’t have a complete picture of how these dynamics affect overall patterns of fertility, the group has known since 2004 that one of these mutations affects almost 40% of all Japanese men, compared with only about 2% of Western European men. “In men of European descent, this variant is a clear risk factor for low sperm count,” says Rozen. “But in Japanese men, its effect is currently unclear.”

### Gene control in depth

**Don’t be fooled** by those neat and orderly textbook illustrations of the inside of a cell. Cellular activity resembles Grand Central Station at rush hour far more than it resembles a game of croquet. Countless proteins and organelles are squeezed together, pitching signals back and forth and back again, carrying out tens of thousands of simultaneous functions.

The control center for all this hustle and bustle is the genome, which stores the operating instructions and responds to extracellular events. It receives its intelligence about the outside world via signal transduction pathways—designated routes by which long lines of proteins pass on a piece of chemical information from the cell surface all the way into the nucleus, bucket-brigade style.

While researchers have gotten pretty good at understanding these pathways, they get stumped at the last few steps, at the precise moment that this pathway concludes in the expression of a particular gene. This is because while evolution has conserved signal transduction pathways throughout many species, it has not conserved the specific gene that the pathway targets. If pathway A in the fruit fly leads to gene B, that same pathway in the mouse might instead lead to gene D.

In a paper published in *Science* last July, scientists in the lab of Whitehead Member Richard Young, led by postdocs Dmitry Pokholok and Julia Zeitlinger, report that a well-known class of proteins called kinases might fill in the dotted lines that connect signal transduction pathways to the genes they regulate.

Kinases are enzymes, proteins whose main function is to catalyze a reaction in other proteins. In particular, kinases target transcription factors, proteins that switch genes on and off. Researchers know that most (though not all) kinases reside in the cell’s nucleus. But they have never paid much attention to the kinases’ exact locations.

Using microarrays to analyze the whole genomes of yeast cells, Pokholok and Zeitlinger decided to track down the location of kinases that reside in the nucleus.

“Many kinases we studied were physically associated with the genome and some of them were located right on the gene itself,” says Zeitlinger. “This surprised us because most molecules associated with gene regulation bind to regions just outside the gene. Here, however, the kinases were located directly on the protein-coding regions of the genes. We never expected that.”

This surprise was pleasant because, unlike other molecules involved in gene expression, kinases are relatively easy to connect back to the signal transduction pathways.
A twist on DNA
The rigors of physics meet the wild and woolly chromosome

By Eric Bender
Illustration by Stuart Bradford

He looks like a biologist.
Sitting at his lab bench, Paul Wiggins works with slightly impatient speed, preparing an experiment on yeast DNA by filling up a tray full of test tubes with genetic markers.

It’s true that the Whitehead Fellow got his doctorate in physics from the California Institute of Technology. The first paper on which he was lead author described interactions between stars and black holes.

But he decided to put the cosmos aside and instead seek ways to quantify biological forces and structures at the molecular level.

“In biology, structure is regulated very carefully; it’s not an accident,” Wiggins says. “Being physicists, we ask, What’s the structure, and how does it affect function?”

“We’re trying to do things very systematically,” he notes. “A lot of theories about this have been based on very circumstantial evidence. And biological models of physical interactions should be more than cartoons. We want to create mathematical models that distill which pieces are important and cut right through to the chase.”

“At the end of the day, numbers matter,” Wiggins emphasizes. “A factor of two in expression of a gene might make the difference between survival and death for an organism.”

ALL WOUND UP, WHERE DOES IT GO?
Most of us first learn to visualize DNA alone in its elegant double-helix form, picturing it floating idly in space.

Well, no. In the teeming chaos of our cells, two meters of DNA are crammed into a nucleus that’s one-hundred-thousandth as wide. Like a string of beads, the DNA double helix is spooled around protein cores to create nucleosomes, and these are packed tightly together to make up a chromatin fiber. Then the chromatin fiber winds in wide loops around protein scaffolding to create a chromosome.

Enter proteins called transcription factors, which control gene expression by binding to DNA regulatory sequences. The binding sites may be next to the gene or far away on the genome. To affect gene expression, distant transcription factors must come into contact with the start of the gene, by bending the intervening DNA.

“Even in the simplest organisms, the genomic space (the length of the intervening DNA) can change the level of gene expression,” says Wiggins. “To understand the processes that the cell uses to convert genetic code to function, you must understand how DNA bends and twists.”

“The physics of DNA bending is very pretty,” he adds, using his belt to demonstrate how DNA twists.

“You basically take a look at every possible configuration. I find these problems very aesthetic because every configuration gets a vote—it’s a bit like a democracy, but some votes count more than others. If a configuration costs more energy it is less likely, and there is a famous formula that relates energy to probability. Essentially you can predict the outcome of a configuration by counting these weighted votes.”

Wiggins, Phil Nelson of the University of Pennsylvania, Cees Dekker of the Delft University of Technology and coworkers have just gathered results from the first experiments to directly and quantitatively measure the elastic properties of DNA on the five-nanometer scale. The researchers studied the bending of a long, random sequence of bacterial DNA with atomic force microscopy. They found that DNA can bend more easily than expected at high rates of curvature.

“We basically claim that the classic theory describing DNA bending fails at the 10-nanometer-length scale, just the scale where biological processes care about it,” Wiggins explains. “Our contribution was to make a direct measurement of the bendability of DNA. But due to the difficulty of pulling off this experiment, there is still skepticism about its results.”
LOCATION, LOCATION, LOCATION
The level of gene expression is strongly affected by the gene’s precise location in the nucleus. “We want to systematically test this idea: How much does where you put something in the chromosome matter, quantitatively?” says Wiggins.

One key challenge is to create spatial measurements of genes in living cells. Joshua Martin, a graduate student in the Wiggins lab, is exploring the structure of chromatin (or rather, for the moment, the structure of DNA within yeast). To do this, he adds fluorescent tags to random locations on the genome. These locations are then sequenced in order to map out where the tags have landed, eventually helping to plumb the depths of gene structure. The work will test theoretical models that Martin has created with Andreas Hochwagen, another Whitehead Fellow, who is collaborating with Wiggins. “It could be that the envelope itself is setting up some kind of territory where genes are kept quiet. You can turn on a gene and watch it being moved into the center of the nucleus, which is pretty cool.”

“It’s not just a ball of wool in there,” Hochwagen says. “But we don’t know much about it. It’s a very basic cell biology problem.”

“No one has mapped out the complete physical locations of an entire chromosome,” says Wiggins. The two Fellows are planning an ambitious effort to take steps toward making that map, and perhaps to eventually extend it to the entire genome.

“In experiments in this field, the results seem to depend on how the material is isolated,” Wiggins says.

“At the end of the day, numbers matter. A factor of two in the expression of a gene might make the difference between survival and death.”

his advisor Jané Kondev of Brandeis University.

Another project in the Wiggins lab looks at the puzzle of heterochromatin regions. These are stretches of condensed chromatin that aren’t expressed most of the time, or are expressed at very low levels, while euchromatin regions are less condensed and more active. Graduate student Brian Ross is making a rigorous study of how the structure of both kinds of regions affects gene expression, warming up with studies in e. coli bacteria.

“There have been a lot of theories about which structural mechanisms drive gene expression, but we’re still really missing the big picture,” Wiggins stresses. “Many things could affect this, such as whether the gene is close to the nuclear envelope or to the center of the nucleus.”

“Genes that are silenced are very frequently found right next to the nuclear envelope—the membrane around the nucleus,” comments Wiggins. “For instance, some researchers have found that chromatin fibers are 30 nanometers in diameter, while others say that it’s 10 nanometers. We’d like to look at things in live cells if possible, and if not, to perturb them as little as possible.”

PHYSICAL POWER
His background as a physicist gives Wiggins a different perspective in thinking about the business of the cell. While biologists often think of it as a chemical reactor, he likes to imagine it as a factory, with proteins as machines on interlocking assembly lines.

As experimental tools and mathematical models evolve, we’re getting much better ways to understand how these incredibly tiny machines crank along. “We can now do experiments where we watch a single molecule do things,” Wiggins points out.

“Many technologies such as x-ray crystallography start out as physics,” he adds cheerfully. “Then they become useful, and they turn into biology.”

Whole lotta shakin’ going on
In everyday life, you and I have a very good intuition for what happens in the physical world,” says Paul Wiggins. “This intuition turns out to be completely wrong for molecular biology.”

Our intuition begins to fall apart at a certain scale, perhaps around one millimeter, the size of a gnat, he suggests. A cell is about 10 microns across, and an enzyme only 10 nanometers (billionths of a meter) wide.

“If what you’re interested in shrinks down enough, suddenly funny things start to happen,” Wiggins says. For one, the pressure surrounding a truly tiny object isn’t standard. That means that “no matter what you look at, if it’s small enough, it moves,” he says. “Ever since we invented microscopes, people have been doing this experiment by accident.”

This is the world of thermal fluctuations in which DNA lives, constantly bouncing against water molecules and everything else around it. And these aren’t soft bounces. At the size of proteins, “atoms are veritable bullets,” Wiggins says. “Enzymes are being hit by things going hundreds of meters per second.”

This is hugely important in biology. “Proteins are continually buffeted by thermal fluctuations, and favorable fluctuations are captured for standard biological functions. But how can you make predictions about the behavior of something that’s being pelted all the time by bullet-speed molecules?”

Fortunately, all these objects are at the same temperature, and “everything is so well-sampled that you can use some powerful mathematical tools that work with random events,” he says. Physicists can examine a molecule, analyze the ways in which it is free to be flexible, and create useful predictions of how these thermal fluctuations will push it around.
Imagine that you’ve been commissioned to map how the entire North American power grid is connected and controlled, to figure out how a unit of power in, say, Kentucky ultimately hopscotches its way to California. You’ve been given a ream of high-resolution satellite photographs that can help reveal the grid. You’ve also developed a few gadgets that can monitor the flow of electrical traffic throughout the continent. But in order to really understand this entire ensemble of power, you must identify every control room, all electrical generators, and the jungle of power lines that connect every McMansion to every office park.

Clearly, the task is insane. There are thousands of control stations, tens of thousands of electrical generators, and millions of miles of transmission lines—all interconnected and overlapping, sometimes looking more like a plate of spaghetti than a feat of engineering. How in the world can you determine the engineering behind this complex network, and how can you be certain that your picture is accurate?

For such a project to succeed, you’d need to create an entirely new technological platform.

Now, shrink the entire continental power grid into the nucleus of a cell, and you’ve entered the world of Whitehead Institute Member Richard Young.

Swap the control rooms for regulatory proteins, the generators for the genes that are the engines of the cell, and the grid for the molecular network that guides our genome throughout the entirety of human development, and you’ll start to grasp the scope of the challenge that faces scientists like Young, researchers who are tackling the circuitry that makes us who we are.

How we’re wired
To uncover the genetic machinery that guides human development, Richard Young is mapping the intricate world of embryonic stem cells

By David Cameron
Photograph by Furnald/Gray
The genome is a seemingly endless terrain whose building blocks—chemicals called nucleotides represented by the letters A, C, T, and G—number about three billion pairs per cell. Identifying how all genes and proteins are networked is like mapping the entire grid from a pile of aerial photos.

But over the last few years, Young’s team of biologists and computer scientists has been pulling it off. A project that began with the yeast genome has now scaled up to human embryonic stem cells, and for the first time we are starting to understand the complex wiring that provides these cells such astonishing elasticity.

And while a comprehensive schematic of the power grid helps engineers troubleshoot breakdowns in the system, an embryonic stem cell counterpart of such a schematic might just provide a roadmap for treating diseases that are currently untreatable.

**THE HUMAN CHALLENGE**

In the winter of 2005, life in the Young lab reached a level of intensity that tested the endurance of some of the most tried-and-true postdocs.

For years, Young’s lab had been working with MIT computer scientist David Gifford to develop a technology platform that could sweep the genome and locate all transcription factors—the proteins responsible for switching genes on and off. While genes contain all the information that defines an organism, transcription factors decide when and where and *how much* of that information gets put into action. Just a few decades ago it would have taken one scientist 100 laboratory years to understand how one transcription factor functions throughout the mammalian genome. This new platform, however, eventually would shrink a century into less than a month.

Having succeeded spectacularly in mapping every transcription factor throughout the yeast genome, the team was now finalizing the first experiments in which they’d scaled the platform up to human embryonic stem cells, a project that also required the computational expertise of Whitehead’s bioinformatics and IT groups. Not only do human cells add many more levels of complexity, but the team was also rushing to get these results published, suspecting competition from other labs.

“We’d be working through the night, taking turns buy-
ing food, eating mostly donuts and Wendy's burgers,” says postdoctoral scientist Matthew Guenther. “I can remember one frantic 5:00 a.m. search for a Dunkin’ Donuts.”

The deadline pressure made the whole project feel like an episode of the TV show 24, recalls fellow postdoc Richard Jenner. “I’d sometimes get home and watch the show,” he recalls. “Seeing the characters dash between each other’s computers as the time ticked away was eerily similar—minus the hidden nuclear warhead.”

The result of these torrid labors was a map of the three principal transcription factors in the embryonic stem cell genome, an initial circuitry diagram of the wiring that makes a stem cell a stem cell. It’s as if they’d scoured the satellite data, located the three most essential power stations on the grid and determined how these stations communicate with each other. It was not the entire network, but for the first time scientists could see, on a global scale, what makes a stem cell unlike any other kind of cell.

UNDER THE HOOD

The three transcription factors that the group located are Oct4, Sox2 and Nanog. Of these three proteins, Oct4 stands out as the primary conductor, the one ultimately responsible for an embryonic stem cell’s most tantalizing characteristic: its ability to become any type of cell in the body, a trait known as pluripotency.

“Oct4 is key to stem cells,” says Richard Young. “In fact, it’s the marker that the scientific community uses to demonstrate that is has a mammalian embryonic stem cell. But the problem was, no one knew what it did.”

Collaborating with fellow Whitehead Member Rudolf Jaenisch and MIT’s David Gifford, the team found that Oct4, Sox2 and Nanog preside over a vast network of developmental genes. Since these genes are responsible for guiding a cell’s fate down a particular developmental path, the primary job of these transcription factors is to keep these networks silent. In that sense, these transcription factors endow an embryonic stem cell with pluripotency not through what they make the cell do, but through what they keep it from doing.

Getting to this point required marrying a tried-and-true lab technique with some modern biotech inventions.

For the traditional technique, called “chromatin immunoprecipitation,” researchers take an unsuspecting embryonic stem cell carrying out its normal functions and instantly freeze it with a few drops of formaldehyde. Adding the chemical essentially stops time in the cells; all molecular activities turned suddenly into inanimate statues. The transcription factors are forever locked onto whatever genes they may have been tinkering with at the moment.

Next, the nucleus of the cell is put through a genomic shredder and broken up into millions of DNA fragments, with the transcription factors still fastened onto their gene segment. The researchers then introduce antibodies to this solution. These antibodies are designed to seek out and latch onto Oct4, Sox2 and Nanog. Each antibody is equipped with a magnetic bead, enabling the scientists to then draw the antibody, the transcription factor and its gene fragment out of the solution once they have latched. Once removed, the antibodies and their magnets are removed from the transcription factors and the gene fragments are labeled with fluorescent tags.

This technique works beautifully for studying one gene, or protein, at a time. But to make the procedure work across the entire genome, Young uses one of biotechnology’s hallmark inventions: the microarray.

ARRAY FOR THE GENOME

For the last ten years, microarrays have been a laboratory staple for anyone studying genomics. The first scientific paper to report use of the array was published in 1995, and in 1997 researchers described using microarrays to profile an entire eukaryotic genome—in this case, baker’s yeast.

Roughly the size of a stick of gum, a microarray is a small glass slide covered with tens of thousands of DNA fragments, each fragment corresponding to a particular gene. Researchers use the microarrays to measure levels of gene expression. The gene pieces covering the microarray all correspond to specific genes in our cells. It is designed this way so that a scientist can place the microarray into a scan-
nizing system with desktop computer software that cross-references the segments to the actual genes. This could tell us, for example, that a particular scrap of DNA on the upper-left-hand corner of the array is taken from gene “A” located on “this particular region” of the X chromosome.

In the Young lab, the fluorescently tagged gene fragments are then released onto the microarray. One characteristic of DNA that makes it so efficient to work with is that it naturally seeks out its complementary sequence. When the gene fragments land on the microarray, they adhere to the matching bits of DNA, and the fluorescent tags allow the researchers to locate where on the array they’ve settled. Once this information is fed into the computer, the scientists can determine where—throughout all 3 billion nucleotide pairs in the genome—each transcription factor was located at the moment formaldehyde froze the cell.

The data is then integrated at David Gifford’s team at MIT’s Laboratory for Computer Science.

“This step is essential given the sheer volume of data that these microarrays produce,” says Young. “We work with thousands of different cells at various time points from a variety of animal models. The Gifford lab is brilliant at integrating all the different data points to build a picture of what the possibilities are. They can assign a probability to any point of the network based on its past performance. This type of information assures that we’re presenting an accurate picture of how the cell is regulated.”

BUT WHO REGULATES THE REGULATORS?
The ultimate hope for many embryonic stem cell researchers is to one day create customized therapies for patients without using a human egg cell. For example, imagine that a patient with leukemia visits a clinic. The clinicians scrape off a skin cell from the patient’s arm, then chemically guide the cell to de-evolve back into an embryonic stem-cell state. The cell is then redirected down a particular developmental pathway into a hematopoietic, or blood, stem cell. These new cells are finally placed into the bone marrow and the patient is cured. And because the new cells are derived from the patient, the patient’s immune system doesn’t react.

Before this becomes a reality, we must thoroughly understand two major aspects of cellular biology: First, we need to know what’s happening during somatic cell nuclear transfer, or therapeutic cloning, in which an egg cell reprograms a donated nucleus. Second is understanding how the genome guides cells down specific developmental pathways.

Rudolf Jaenisch and other scientists around the world are pursuing the first task, producing preliminary proof-of-concept experiments. “We know that when an egg cell reprograms a donated nucleus during nuclear transfer, it doesn’t work some kind of magic,” Jaenisch says. “It’s a biochemical process, one that we can learn, and one that we can most likely reproduce in the lab.”

Young and his team are working on the second aspect, focusing on the mechanisms that guide a stem cell into forming differentiated, mature tissue.

“We want to understand the wiring in an embryonic stem cell so that we can know exactly what transpires in that cell as it develops into a neuron or a blood cell or a pancreatic islet,” he says.

This spring, Young, Jaenisch and colleagues reported in the journal Cell how a network of developmental proteins called polycomb controls the embryonic stem cell genome.

While all cells—including stem cells—share the identical genome, each cell type is only granted access to a select group of genes specific to that cell. All other genes are kept behind a firewall. Polycomb ensures this by tagging the genome with a chemical marker that prevents access to genes. However, polycomb had never been thoroughly studied in embryonic stem cells before.

The researchers reported a significant overlap of polycomb and the key transcription factors Oct4, Sox2 and Nanog in the human embryonic stem cell genome. These parts of the genome where the transcription factors and polycomb converge encode genes involved in the control of most of human development. This suggests that these scientists have found themselves at the core of the regulatory circuitry that controls human development. Because there are close links between the control of development and disease, the discovery places us closer to an understanding of the regulatory dysfunctions involved in many diseases.

Perhaps ten years from now, Young suggest, we’ll pair our new knowledge of regulatory circuits with therapeutics that can act on those circuits when they break down.

“We may not cure Alzheimer’s in the near future,” Young says, “but I’m confident that as we continue to unravel and map human wiring, we’ll be shocked at the range of new possibilities for attacking disease.”

That’s what lies ahead as we continue to map the vast power grid tucked inside of every one of our cells. W
It's 2:00 on a Sunday afternoon, and the stem cells are hungry.

Maya Mitalipova, director of the Institute's Stem Cell Facility, drops whatever she's doing, exits her Cambridge apartment, and heads over to Whitehead. She hurries to the refrigerator in her lab.

And there they are, thousands of them, clustered on Petri dishes in tiny groups of a few hundred.

Gently, she takes out and sets down the dishes, opens up a second fridge and removes a vial containing the occupants' favorite food: a formula whose primary ingredient is calf blood. They love the stuff. She warms the vial in a water bath for 15 minutes. Then, pipette in hand, she fills the dishes.

“If I do anything different in their feeding schedule, I may lose 90 percent of a colony,” says Mitalipova. “I get a dramatic reaction if I ignore any aspect of them.”

Mitalipova speaks from years of experience. Before coming to Whitehead, she was already established as a leading expert in culturing and maintaining stem cells at the University of Georgia. And before that, she had isolated stem cells that are now part of the so-called presidential lines.

While maintaining embryonic stem cells may feel a lot like running an intensive-care unit, the cell itself is no more a “patient” than a particle of skin. A stem cell, after all, is just a cell: a membrane and a nucleus buffered by cytoplasm.

But while a skin cell is robust and can live happily on a growth medium with minimal attention, stem cells require an exhausting degree of care giving.

The reason is simple: a skin cell has completed its developmental journey. It can never be anything but a skin cell. It will divide and replicate itself only when it needs to. Otherwise, it simply sits back and drapes your bones.

An embryonic stem cell, on the other hand, is at the starting gate of development. It is pure potential. It hasn’t been assigned a particular fate yet, but it’s dying to get to work and become that liver or brain or hair follicle—anything but a stem cell.

For scientific projects, though, these cells are only valuable to the degree that they are kept from differentiating.

Here’s the dilemma for people in Mitalipova’s position: How do you give an embryonic stem cell everything it needs to thrive, yet keep it from doing the very thing it wants most of all to do?

According to Mitalipova, with great difficulty.

“Timing is critical,” she says. “Neglecting them for just one day can have dire consequences.”

Yes, this is as onerous as it sounds. Seven days a week, someone must attend to the cells. If both Mitalipova and her technical assistant, Ping Xu, need to be away for a few days, they must freeze the cells—an option which is the absolute last resort. “Once you freeze the stem cells, they take two weeks to thaw,” she says.

CHECKING THEIR IDs

In addition to the daily feedings, Mitalipova needs to continually propagate the stem cell lines so that other researchers in the Institute can use them.

When stem cells are placed in the Petri dish, they immediately divide and start forming clusters, or colonies. For the first few days, this is exactly what you would want.

But once day four or five approaches, it’s time to start getting nervous. “Around this time, each colony has about 500 cells,” says Mitalipova. “Any day each cell will start signaling its nucleus saying, ‘I’m ready to go!’ and it will start trying to develop into some other kind of cell.”

She can tell this is happening simply by taking a good look. Stem cells are perfectly round with dome-like surfaces. When they differentiate they become irregular, less like a spherical drop of water and more like an ink blot.

For reasons that aren’t yet clear, the size of the colony, more than anything else, determines whether or not this happens. When a colony reaches the 500-cell threshold, Mitalipova performs a technique called “passaging.” Here, she adds an enzyme that loosens the cells from their feeder bed, and then she breaks the colony apart into groups of anywhere from 10 to 100 cells. Each of these smaller groups then forms its own colony that will, in about four days, reach the 500 mark, when she will then need to repeat the process. And so on.

“It’s important that I don’t separate them to less than 10 cells,” she says. “Unlike mouse embryonic stem cells, the human cells need cell-to-cell contact in order to survive.”

Life for Mitalipova won’t be getting easier any time soon. Seventeen new lines of human embryonic stem cells recently arrived at the Institute.

“We need to make millions of clones of all the different lines and freeze them. We’ll be feeding them constantly, examining the colonies, measuring their genetic profiles.” She sighs.

“I won’t have a single day off for the next five months.”

The care and feeding of stem cells

What do embryonic stem cell facilities and intensive-care units have in common?

By David Cameron
Photographs by Furnald/Gray
After the passing, Maya Mitalipova places stem cells in solution in a test tube.

Technical assistant Ping Xu removes embryonic stem cells from the incubator.

The “passaging” procedure breaks down large colonies into small ones to prevent differentiation.

After the tubes are treated in a centrifuge, the cells will be ready to return to their Petri dishes.
For the last 100 years, scientists, teachers and parents have been relying mostly on lawyers to keep religion out of public school science classes in this country. So far, the lawyers have been doing a pretty good job.

But the burden is shifting to the scientists themselves, say experts involved in recent cases defending public school science curricula from anti-evolution revisions. “The buck stops with university professors,” says Eugenie Scott, executive director of the National Center for Science Education in Oakland, California.

It is tempting for scientists to insist that creationist perspectives should not be dignified with a response, says Richard Katskee, assistant legal director of Americans United for Separation of Church and State and one of the four principal lawyers in last year's rout of the Dover, Pennsylvania, school board mandate to teach intelligent design. “We're talking about stuff that is intentionally designed to deceive kids,” says Katskee. “It's a national phenomenon that will have a real and palpable impact on the future of science education will be undermined. The voices of serious scientists speaking up will make the difference.”

Scientists have to speak out,” says Whitehead Member Harvey Lodish. “Biology is the study of evolution. It is the history of life going back four billion years. Evolution is not an abstract concept. It is a working tool. It is a powerful set of arguments we use all the time to be able to infer from experiments in yeast, rats and mice what humans are like.”

Two years ago, in his role as president of the 11,000-member American Society of Cell Biology, Lodish petitioned the governor and the state education board in Ohio, his home state, to reject a new 10th-grade model science lesson plan that included components of intelligent design. In February 2006, the Ohio board finally removed the religious-based material from the science curriculum.

THE EVOLUTION OF CREATIONISM

The strong and comprehensive court ruling in the Dover case last December marks the end of the most recent resurgence of anti-evolution teaching activity in the United States, wrote George Annas, professor of law and public health at Boston University, in the May 25 New England Journal of Medicine.

But if the past offers any precedents, the same concepts will be repackaged under different names.

Teaching evolution was first outlawed outright in 1925, provoking one of the most famous trials of the 20th century, in which John Thomas Scopes was tried and convicted. In 1968, the U.S. Supreme Court overturned a similar Arkansas law as unconstitutional for furthering a religious purpose. Next came state laws in Arkansas and Louisiana to balance the teaching of evolution with “creation science,” a spin also ruled unconstitutional in 1982 and 1987, respectively, for the same First Amendment violation.

The third wave was intelligent design (ID), which allows for limited evolution within species, including antibiotic resistance, but is silent about other creationist claims, such as the earth's age. ID posits that life is too complex to have evolved from common ancestors who crawled grudgingly out of the primordial soup.
“In a way, it is a classical example of evolution at work,” Steven Gey, a leading scholar on religious liberties and free speech at Florida State University, told an audience there in May. “ID is creationism that evolved in response to a series of legal decisions that said creationism is not going to fly under the First Amendment.”

Now that the Dover decision has effectively exposed ID as a rebranded form of creationism, Annas, Gey and others predict a fourth strategy with a seductive campaign to “teach the controversy.” Never mind that the controversy is largely manufactured by proponents of creationism and ID.

“It’s one of the three pillars of creationism: Evolution theory is in crisis, evolution and Christianity are incompatible, and it’s only fair to balance evolution with something,” Scott says. “The fairness argument is incredibly powerful in a country like ours.”

The only major pending case now involves the Cobb County, Georgia, school board. A federal district court had ruled that textbook stickers describing evolution as “a theory, not a fact” violate the First Amendment. On May 25, the 11th Circuit Court of Appeals remanded the case back to the district court to sort out problems with the record. That may mean additional court hearings on the evidence and a possible new ruling. “If this goes the wrong way, it will be the first case we’ve ever lost between scientists and the fundamentalist objections to scientific data,” notes Gey.

The new arguments against evolution are increasingly framed in terms of molecular biology, cell biology and information technology—languages that evolutionary biologists do not speak as well, says Kenneth Miller, a cell biologist and biochemist at Brown University and expert witness in the Dover case.

Miller first became involved in the issue when he accepted a student challenge to debate the founder of the Institute for Creation Research on campus 25 years ago. As he researched the opposing position, he became infuriated with the deceptive misrepresentation of scientific evidence. Worse, the Roman Catholic researcher realized, “what they wanted to stick in the science classroom wasn’t God per se, but it was their view of religion, not mine.”

PEACEFUL COEXISTENCE
In his book Finding Darwin’s God, Miller defends his view that belief in God and evolutionary theory can coexist peacefully.

“There are many levels of understanding of the causal ingredients of almost anything that happens in our universe,” says John Haught, a theologian at Georgetown University. “You don’t have to see science as in any way competing with fundamental religious positions.”

Haught urges scientists to keep religion out of the science classroom. “There are prominent science thinkers and writers who have themselves unconsciously folded evolutionary science into a world view that nature is all there is, so there cannot, a priori, be any other explanations,” he said. “The irony is that this sabotages and subverts the whole mission of scientific education.”

The most important role scientists can play is to teach evolution better in classes and communicate the accumulating pieces of evidence and the nature of science more clearly and more often to the general public, say lawyers, scientists and teachers. And if a crisis arises in their backyard, scientists need to speak out.

“You are not trying to convert partisans on the other side,” Miller says. “You are trying to reach out to the great middle ground of American people who, if they fail to support science, ultimately threaten the scientific enterprise. If we in the scientific community don’t provide the information, the American people won’t have the chance to come to the right decision, and it will be our fault.”

How to jump in
Individual scientists can strengthen the understanding of evolution and science itself, says Glenn Branch, deputy director of the National Center for Science Education (NCSE). Here’s what he suggests:

- Teach your science well. Students become the informed citizenry that understand the evidence for evolution and support high-quality science education.
- Inform the public about your research. That helps to explain the evolutionary concepts behind it.
- Do your homework. Uninformed responses to creationism can do more harm than good. For example, presenting the evolution debate as science versus religion may compel people to choose the religious side.
- Write an opinion piece. Your words may stiffen the backbone and strengthen the defense of a teacher who is under pressure to inject religion into her science class.
- Team up with teachers, lawyers, clergy and anyone else who can address what else is at stake, such as quality of education and the separation of church and state.
- Organize outreach activities, such as public lectures or workshops for teachers and other community leaders.
Few things put us in a worse mood than sitting in traffic. But when a similar kind of traffic jam occurs in our neurons, the consequences are far more dire. Parkinson’s disease can set in, and when it does, there’s no turning back.

In general, statistics for neurological disorders are grim. More than a million Americans suffer from Parkinson’s disease alone—a number that is expected to soar over the next few decades as the population ages. No current therapies alter the fundamental clinical course of the condition.

A recent advance in our understanding of this condition, however, makes researchers a bit more optimistic about our prospects for eventually treating patients more effectively.

Scientists at Whitehead Institute, in collaboration with colleagues at several research centers, including the University of Missouri’s School of Biological Sciences, have identified a key biological thoroughfare that, when backed up, causes Parkinson’s symptoms.

Even more importantly, they have figured out how to repair the traffic flow and restore normal neurological function in certain animal models.
“For the first time we’ve been able to repair dopaminergic neurons, the specific cells that are damaged in Parkinson’s disease,” says Whitehead Member and Howard Hughes Medical Institute Investigator Susan Lindquist, senior author in the paper appearing last June in Science.

This research began back in 2003, when Tiago Outeiro, a graduate student in Lindquist’s lab, described using yeast cells as “living test tubes” in which to study Parkinson’s. A Science paper reported that when a Parkinson’s-related protein called alpha-synuclein was over-expressed in these cells, clumps of misshapen proteins gathered near the membrane, and in many cases the cells either became sick or died.

This paper was of particular interest because the findings were arrived at via yeast cells, a rather unlikely model organism for brain disease.

Aaron Gitler and Anil Cashikar, postdoctoral researchers in the Lindquist lab, decided to follow up by asking a simple question: Is it possible to rescue these cells when an overexpression of alpha-synuclein would normally make them sick?

They began with an array of yeast cells in which each cell over-expressed one particular gene. This array, prepared by scientists at the Harvard Institute of Proteomics, covers the entire yeast genome. All cells were also infected with alpha-synuclein. They reasoned that if they identified genes whose over-expression rescued a cell, that would tell them something about how alpha-synuclein made the cell sick in the first place.

Most of the proteins that they identified pointed to a pathway that involves two cellular organelles, the endoplasmic reticulum (ER) and the Golgi complex. The ER is the cell’s protein factory, where proteins assume their requisite shapes. Once a protein has properly folded, it is trafficked over to the Golgi, where it is fine-tuned and further prepared for its designated task.

Working with Antony Cooper from the University of Missouri, Kansas City, Lindquist’s team demonstrated that when alpha-synuclein becomes mutated and clumps at the cell surface, it drags away a protein that eases transport between the ER and the Golgi.

“This protein is the traffic cop for the ER-Golgi route,” says Lindquist. “Once it’s gone, the molecules start backing up at the ER.” It is this cellular traffic jam that causes cell death.

This isn’t just a general toxic effect caused by any misfolded protein. It is specific to alpha-synuclein, the protein associated with Parkinson’s disease.

REPAIRING NEURONS

“All this was done in yeast,” says Gitler. “Our next goal was to find what this told us about actual neurons.”

If mutations of alpha-synuclein dragged the ER-Golgi protein away from doing its job of directing traffic, as the yeast research indicated, then cell death might be averted simply by increasing the levels of this transport protein—that is, by adding extra traffic cops to the scene.

Working with colleagues at the University of Pennsylvania, the University of Alabama, and Purdue University, the consortium tested this hypothesis in the fruit fly, C. elegans worm and in neurons culled from rats—all of which had alpha-synuclein-induced Parkinson’s symptoms. In every case, symptoms were reversed by increasing levels of the trafficking protein.

“We tried this a number of different ways, from creating transgenic animals that naturally over-expressed this protein, to injecting a copy of the gene for this transport protein into the neurons through a gene-therapy technique,” says Gitler. “The results were the same. Cell death ceased, and the neurons were restored to normal health.”

“Protein folding problems are universal, so we hoped we could use these simple model organisms to study something as deeply complex as neurodegenerative disease,” says Lindquist, who is also a professor of biology at MIT. “Most people thought we were crazy. But we now not only have made progress in understanding this dreadful disease, but we have a new platform for screening pharmaceuticals.”

“This gives a whole new direction for understanding what’s been going wrong in these patients, and for considering much better strategies for treating people,” says Cooper.
The brains of patients with neurodegenerative diseases contain deposits of aggregated proteins.

Neurons from these patients contain clumps of a protein called alpha-synuclein, but our understanding of the role these clumps play in disease is poor.

Baker's yeast is the simplest and easiest to manage of all eukaryotic cells (which include a discrete nucleus).

The mind of yeast

How can individual yeast cells yield insights into complex diseases of the brain?
Researchers in the Lindquist lab engineered yeast cells to produce high levels of alpha-synuclein and observed the process by which the protein clumps at the cell surface (see page 14). Just as in neurons, this caused cell death in the yeast.

Next, they placed alpha-synuclein-expressing cells into a series of plates. Each well on the plates contained a different yeast gene that, when introduced to the cells, became over-expressed. This approach allowed scientists to identify genes that can reverse the toxic effects of alpha-synuclein.

A group of genes all pointed to a pathway in the cell that illuminated how mutations of alpha-synuclein ultimately can cause cell death. This discovery was corroborated in fruit flies, worms and rat neurons, giving a new drug target for this condition.
Though the first multi-lens microscopes were built about 400 years ago, and the devices have been a mainstay of biological research nearly as long, scientists couldn’t harness their full power until digital photography made it possible to leverage computational tools for image analysis. Researchers now use automated microscopy systems to take thousands of pictures of cells, and software to mine the pictures for patterns.

Paul Matsudaira, Whitehead Member and director of the Whitehead-MIT BioImaging Center, believes this new form of bioimaging will play a powerful role in the emerging field of systems biology.

“Bioimaging will help scientists probe the complex relationships between genes, proteins, cellular components and physiological systems,” he says. “Systems biologists need tools to collect and make sense of mountains of data.”

By the end of this spring, Whitehead postdoctoral fellow Robert Wheeler, who generated more than 100 gigabytes of data this spring by taking pictures of yeast cells, had identified proteins that mask fungal pathogens from our immune system, shedding light on the action of certain antifungal drugs.

Welcome to the era of digital microscopy, defined by quantitative analyses rather than qualitative observations.

Digital microscopy and powerful software are turbocharging systems biology

**By Alyssa Kneller | Photographs by Furnald/Gray**

The new age of bioimaging

“ANYONE CAN TAKE PRETTY PICTURES,” says research scientist James Evans, who oversees the imaging arm of MIT’s Computational and Systems Biology initiative. “The challenge lies in extracting information from them and telling a story.”

Take the case of Whitehead postdoctoral fellow Robert Wheeler, who generated more than 100 gigabytes of data this spring by taking pictures of yeast cells. A computer program scanned all the images and identified proteins that mask fungal pathogens from our immune system, shedding light on the action of certain antifungal drugs.

Welcome to the era of digital microscopy, defined by quantitative analyses rather than qualitative observations.

Though the first multi-lens microscopes were built about 400 years ago, and the devices have been a mainstay of biological research nearly as long, scientists couldn’t harness their full power until digital photography made it possible to leverage computational tools for image analysis. Researchers now use automated microscopy systems to take thousands of pictures of cells, and software to mine the pictures for patterns.

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But it’s still early days for digital bioimaging. Few labs use quantitative characterizations of pictures to advance their research. Microscopy pioneers blame a variety of factors, including the high cost of cutting-edge microscopes and data storage. (The Whitehead-MIT center can store more than 40 terabytes—about 10 million pictures taken with a consumer digital camera.)

Two of the biggest challenges, however, are the need for
better image-analysis software, and for a greater awareness among life scientists about how these new tools can benefit research.

SOFTWARE WITH A SHARPER FOCUS
Mining a gigabyte of data is no picnic. Imagine sifting through thousands of photos in search of cells that are dividing, or cells containing a specific fluorescently labeled protein. The image-analysis software that’s needed doesn’t materialize on its own. Scientists must write or tweak computer programs virtually every time they design experiments involving advanced microscopy.

“Bioimaging software is more powerful and easier to use than it was a few years ago, but it’s not plug and play,” says Matsudaira.

Biologists didn’t invent image-analysis software. The federal government first used computer programs to locate missiles, tanks and ships in photos taken by satellites during the late 1950s.

During the last decade, researchers began applying the descendants of these early programs to biology, training computers to recognize cellular components. Bioimaging software improved as scientists modified beta versions to meet their needs.

A growing group of researchers, including postdoctoral fellow Anne Carpenter of Whitehead Member David Sabatini’s lab, contribute to this iterative process.

Dissatisfied with existing software, Carpenter initiated collaborations with scientists in several labs at Whitehead and MIT and created a program that allows them to test the effects of many genes on cell size and appearance. “Coming from the biology side, I knew what the software needed to do, and I knew enough programming so I could get started on the project,” she says. “I turned to computer science graduate students for help when I got stuck.”

The software is freely available and can analyze a wide variety of biological images, but investigators must adapt it for specific cell types and conditions.

When Matsudaira and former Whitehead Member Peter S. Kim (now president of Merck Research Laboratories) first conceived of the Whitehead-MIT BiOImaging Center in the late 1990s, they recognized the importance of customized software. The first hires were Evans (a molecular cell biologist who specializes in imaging), computer systems engineers and other scientists with image-analysis and computation expertise.

“When scientists approach me with projects, I can’t pull out a cookbook and tell them what to do,” says Evans. “We work together to identify the parameters and stitch together the appropriate tools, The technology develops through these collaborations.”

Matsudaira believes the pace of development will increase as biology educational programs place greater emphasis on computation. Graduates will feel comfortable working with complex equations and enhancing software on their own.

SPREADING THE WORD
The number of labs involved in digital bioimaging will also grow as scientists realize how it applies to their work. Professors associated with the Whitehead-MIT center expedite the process by designing demos to educate their colleagues. Each demo explores a real biological process, letting researchers answer interesting questions and publish their methods and findings in peer-reviewed journals.

Douglas Lauffenburger’s group, for example, used imaging to carefully map the movement of breast cells after over-expressing a receptor associated with cancer (see page 22). The study, which could have implications for drug development, appeared in Biophysical Journal this August.

“Basic cell biology and pharmaceutical industry efforts currently don’t place much emphasis on detailed, quantitative characterizations of cell motility,” says Lauffenburger, who is director of MIT’s Biological Engineering Division. “It’s up to bioengineering labs like mine to demonstrate how important this is.”

That importance will only rise as the scale of experiments increases in many labs. Automated microscopy and image analysis should fare well as “big science” progresses.

The following pages highlight the work of researchers at Whitehead and MIT breaking new ground in bioimaging.

Seeing the true image

Digital imaging holds great promise for research advances—and for abuse. Nicki Watson, who manages the W. M. Keck Biological Imaging Facility at Whitehead, trains scientists to avoid common pitfalls.

“It’s very easy to take a digital picture of what you want to see, but you need to discipline yourself to take a picture of what’s actually there,” she says.

Scientists sometimes run into trouble if they make mistakes while preparing specimens, alter images by hand and/or interpret them incorrectly. Photo-editing software, for example, allows them to easily eliminate noise and enhance elements of interest. But what exactly is “noise”? In some cases, the background of a photo contains relevant information.

“Scientists have an obligation to report how they’ve manipulated data,” says Whitehead-MIT BioImaging Center director Paul Matsudaira. He also urges scientists to avoid being led astray by image-analysis software. They should make sure such programs function as intended and keep in mind they could be missing some interesting patterns.

www.whitehead.mit.edu
Interest in facial recognition software increased in recent years. Computers can “recognize” individuals’ faces, as they can identify individuals’ fingerprints. Postdoctoral researcher Jason Moffat of David Sabatini’s lab employs similar technology to identify genes that could be involved in mitosis (cell division).

“In Jason’s experiment, programs sorted pictures or ‘portraits’ of cells by examining ‘features’ such as cell shape and DNA distribution,” says Sabatini.

Moffat wanted to apply image-analysis techniques to identify genes required for cell growth and division. He also wanted to show scientists how to use a new tool developed by a team at Whitehead and the Broad Institute. The collaborators manufactured special viruses, designing them to infect cells and silence specific human and mouse genes. The team built thousands of unique viruses, and Moffat decided to use the resulting library to perform a massive gene-knockout experiment.

After weighing several potential projects, he chose to focus on mitosis. He planned to screen more than 1,000 human genes to determine which ones play a role in the cell division process. Scientists had already identified some of the genes involved, giving him a basis for comparison.

Next, Moffat turned to a Cellomics ArrayScan automated fluorescence microscopic-imaging system designed for high-content screening. He prepared hundreds of plates, placing human cells in thousands of wells. Each well received a different virus. After waiting for the vectors to work, the microscope took pictures of each well.

But what did those pictures tell Moffat about cell division? After allowing the viruses to knock down their intended target genes, he added fluorescent labels for three components of the cells—DNA, actin (to outline the cell shape) and a structural protein that is detectably modified through mitosis green.

He used a similar labeling scheme in the bottom two images, though he dropped the actin label and added one for a structural component of microtubules.

“The literature on mitosis in mammals is still murky. We’re fairly confident that other labs will eventually confirm the role of these genes.” —JASON MOFFAT

Faces of mitosis

Postdoc Jason Moffat silenced particular genes in human colon cancer cells and then fluorescently labeled several components of the cells to expose cell division problems. In the top four images, he labeled DNA blue, actin red (to outline the cell shape), and a structural protein that is detectably modified through mitosis green. He used a similar labeling scheme in the bottom two images, though he dropped the actin label and added one for a structural component of microtubules.
A woman lies on the beach on a warm summer day, bathing her skin in ultraviolet light. A man takes a drag on a cigarette, filling his lungs with smoke. Although DNA-damaging agents such as these bombard our cells every day, proteins heal most of the injuries. A team of scientists developed a high-throughput system to probe components of this DNA repair kit during the summer of 2005.

MIT's Computational and Systems Biology Initiative (CSBi) supported Professor Carlos Ríos-Velázquez, who took a three-month sabbatical from the University of Puerto Rico at Mayagüez to spearhead the effort as part of an outreach program for minority faculty. He traveled to Cambridge with graduate student Josué Malevé-Orengo and undergraduate Alana Toro-Ramos, and worked in the labs of Rebecca Fry and Leona Samson.

The team decided to map the concentration and location of all the yeast proteins that respond to DNA-damaging agents. Each yeast cell contains about 4,000 genes that code for proteins, and Ríos-Velázquez wondered how many play a "detoxifying" role. His team tapped into a yeast library to investigate their functions.

Each “book” in the library consists of a yeast cell with a modified gene for a particular protein. When produced, the protein glows green. Ríos-Velázquez resolved to screen the entire yeast genome by exposing each book to a DNA-damaging agent and photographing the results.

Ríos-Velázquez has not yet completed the screen, but his team tested the procedure and determined it will work. (They are using a Cellomics Array-Scan at the Whitehead-MIT center to automate the experiment, though the microscope works best with mammalian cells, which are bigger.) Members doused two strains of yeast cells—books corresponding to well-studied proteins—with a potent DNA-damaging agent. Before and after shots revealed that the proteins responded as expected. One protein moved from the nucleus to the cytoplasm, and the level of the other protein increased.

"Soon we’ll be able to see how the entire cell reacts to a specific environmental change,” says Ríos-Velázquez. “We’ll have a global view of the response.”
Tracking cells on the move

A cancer cell breaks away from a tumor and moves to a new location, where it divides. Mystery shrouds the mechanics of this process, which is known as metastasis. But researchers at Whitehead and MIT pieced together part of the puzzle last spring by making movies and modeling the movement of the “actors.”

“We wanted to understand breast cancer better, so we decided to study a protein called human epidermal (HER2) because it’s over-expressed in 20 to 30 percent of breast cancers, and it’s correlated with poor prognosis and increased metastasis,” explains MIT graduate student Neil Kumar of Douglas Lauffenburger’s lab.

Kumar grew two lines of human breast cells—one with normal levels of HER2 and one with high levels—on the surface of a gooey matrix in 96-well plates. He scraped the colonies of cells to create “wounds” and then loaded the plates into a Cellomics Kinetic-Scan automated imaging system designed for high-content screening of cells in motion.

This microscope took pictures every 15 minutes for approximately 15 hours.

Kumar worked with MIT graduate student Hyung-Do Kim and Whitehead post-doctoral fellow Muhammad Zaman (formerly a researcher in Paul Matsudaira’s lab and now an assistant professor at the University of Texas at Austin) to quantify wound closure in the resulting time-lapse movies.

“We’re engineers, so it is a natural goal to characterize the behavior of this system quantitatively and mathematically,” says Kim.

A quick review of the movies revealed that wounds closed faster in cells containing more HER2. But the team moved beyond this crude observation by writing computer programs that tracked the speed and direction of individual cells.

“Think of a cell as a car moving from point A to point B,” suggests an animated Kumar. “If the driver steps on the gas, the car will get to point B faster. If the driver straightens the steering wheel and takes a direct path, the car will also get to point B faster.”

The cells with high levels of HER2 stepped on the gas mildly, but more importantly dramatically straightened their steering wheels. Some of the matrices contained materials that enhanced this effect, as HER2 is a surface receptor that responds to environmental cues. Biophysical Journal published these results in August.

“These findings could have significant implications for development of anti-cancer therapeutics,” says Lauffenburger. “We’ve uncovered a level of cell motility regulation that wasn’t fully appreciated in the past.”

Lauffenburger likens the discovery to one that scientists made while modeling cell growth. They began by measuring changes in the net number of cells. Subsequent work showed they were glazing over two distinct processes—cell division and cell death—regulated by a variety of proteins. Pharmaceutical companies now use some of the revised models to more accurately predict the effects of potential cancer treatments.

Left: Wounds close more quickly when breast cells (such as those shown in the bottom row) contain high levels of the protein human epidermal (HER2).

Right: Researchers tracked individual cell movement to better understand migration characteristics. Cells in the bottom plot contained higher levels of HER2, and moved straighter and faster than cells in the top plot.
In Neil Kumar’s study, cells crawled across the surface of a matrix, traveling in a single plane. Muhammad Zaman wondered how they would behave in the middle of this material. Would they move at the same speed and in the same direction? Would they stay the same shape? He developed a model based on a series of calculations about what the two-dimensional model missed, and designed an experiment to test it.

Like Kumar, he grew two lines of cells—one with normal levels of a protein associated with metastasis and one with high levels. The projects then diverged.

Zaman worked with prostate cells rather than breast cells. He dropped the prostate cells into a thick, soupy matrix, and placed them under a special confocal microscope, which divided each specimen into virtual slices. A laser scanned the slices separately at regular intervals, generating a new stack of images every 15 minutes.

Zaman collected these series of 3D images for months. While the Cellomics KineticScan microscope in Kumar’s study photographed 96 samples at a time, the confocal microscope scans just one at a time.

But the hard work paid off. After quantifying the movement of the cells, Zaman found that they behave completely differently in 3D, confirming his hypothesis. The online early edition of *Proceedings of the National Academy of Sciences* published the results in July.

“Two-dimensional models ignore the obstacles that cells face in their natural contexts,” explains Zaman. “In 3D, cells move through a thick jungle of fibers or ‘vines’ that hinder forward progress.”

Cells must either squeeze through or chop up these putative vines to get anywhere. As a result, they move slower in 3D.

In an interesting twist, all cells need at least some vines to move, as they stick onto the “branches” with adhesive-like proteins called integrins and pull themselves forward. When Zaman reduced the adhesive-ness, in a manner analogous to certain anti-cancer drugs, the cells moving across the top of the forest canopy (in two dimensions) needed a greater number of vines to keep up their pace, while cells plowing through the jungle needed vines chopped to maintain the same speed.

Though he uncovered key differences in the way cells behave in two and three dimensions, he also discovered a similarity. In a given setting, prostate cells with high levels of the receptor associated with metastasis always moved faster than normal cells. But the physical and chemical composition of the matrix reduced the persistence of their movement in 3D.

“If you plunk a car down in Cambridge, step on the gas and drive around in circles, you’re still stuck in Cambridge. If a cancer cell does the same thing, then it can never start a tumor in a new location,” says Lauffenburger. He believes pharmaceutical companies will eventually adopt 3D models to study how drugs affect metastasis.

**Entering the third dimension**

*Two-dimensional models ignore the obstacles that cells face in their natural contexts. In 3D, cells move through a thick jungle of fibers that hinder forward progress.*

—MUHAMMAD ZAMAN

Researchers tracked the movement of human prostate tumor cells in a three-dimensional matrix.
Mention fungal cells, and Whitehead postdoc Robert Wheeler thinks of M&Ms. “Imagine a fungal cell as a sugar-coated pathogen,” he says. “The organism is encased in a hard, white ‘candy’ shell, which is covered with a thin layer of sweet ‘paint.’”

The layers, which consist of complex sugars called beta-glucan and mannan, hint at an evolutionary arms race that plays out over millions of years. First, immune systems detect beta-glucan. The pathogens can’t get rid of the protein because it serves a critical structural purpose, so they hide it with a coat of mannan.

“This may very well be one more tactic in the ongoing hide-and-seek game between our immune systems and pathogenic fungi,” says Whitehead Member Gerald Fink, who is Wheeler’s advisor.

The pair worked with baker’s yeast (a fungus) to identify the genes associated with the cloaking device. They began with a library of yeast strains. Each one contained a mutation on a specific gene. Wheeler placed the strains in 96-well plates and stained them with substances that bind to beta-glucan and mannan. He prepared 52 plates, as he wanted to screen all 4,080 yeast genes, and loaded them into a Cellomics ArrayScan. He instructed the microscope to take pictures, find cells and quantify beta-glucan exposure for each strain.

A computer program processed the photos, locating cells by searching for the mannan stain. It drew a circle around each cell and tried to detect beta-glucan inside. When the circles glowed bright, Wheeler knew he’d poked holes in the cells’ outer coat of paint. When the circles remained dark, he concluded the paint was intact. Thus he pinpointed the genes responsible for maintaining the yeast’s mask by disabling them, one by one, and then monitoring the result.

“I wouldn’t have tackled this project without automated imaging,” says Wheeler. “The Cellomics equipment allowed me to conduct 96 experiments quickly at the same time.”

His findings, which appeared in the April issue of the journal PLoS Pathogens, have implications for drug development. Pharmaceutical companies could target the genes that control the mannan layer. Wheeler hopes they will create more drugs like caspofungin, which he showed can boost immune response by unmasking fungi.
A narrow tube of cells forms in a zebrafish embryo just hours after conception. As the cells divide, the walls of the tube bend and fold, creating cavities that fill with fluid. Postdoc Jennifer Gutzman uses a spinning-disc laser microscope to take pictures of these elaborate structures, which eventually mature into brain cavities. She makes movies of the developing brain, capturing the movement of individual cells.

“The movies shed light on our own minds, as the early stages of development are virtually identical among fish and humans,” says Gutzman, who works in Whitehead Member Hazel Sive’s lab.

Scientists have already identified dozens of proteins that control neural patterning by mutating genes and watching for brain defects. Gutzman decided to characterize some of these defects in detail at the cellular level. She began by tracking individual cells in normal (mutation-free) embryos to establish a reference point.

Preparing the specimens took a huge chunk of time. She injected a fertilized egg with a piece of RNA encoding a green fluorescent protein that binds to cell membranes and outlines every individual cell. She let the zebrafish develop overnight, and in the morning, just before each embryo turned 18 hours old, she placed jelly-like material on a slide and used a pipette to pluck out a piece, wedging the young critter in the resulting hole. She added water and a covering before racing to a spinning-disc laser microscope equipped with a heater (to keep the specimen warm) to conduct her experiment during the correct phase of development.

Next came the imaging. She programmed the microscope to take virtual slices of the developing embryo every five minutes, creating a three-dimensional movie. In the past, scientists used scanning laser confocals to accomplish this task, but they often damaged specimens because they took so long to generate pictures.

“To create a single stack of 200 images, scanning laser confocals bathe specimens in light for about 15 minutes,” says Gutzman. “A spinning-disc laser confocal can accomplish the same task in just 20 seconds.”

The new technology allowed her to keep specimens alive long enough to create the perfect four-hour movie. Next, she will make movies of mutant embryos and compare them to the originals.

“It is extraordinary to be able to look at the developing brain as it forms and see what cells are doing,” says Sive. “This is an enormous leap in understanding mechanisms underlying brain development.”

David Cameron contributed to this story.
Fixing K-12 biology

Education expert Melanie Barron calls for an inquiry-based curriculum

Worried about the state of biology education in U.S. public schools? Keep worrying.

In May, the National Assessment of Educational Progress released its Science Scorecard, revealing that while modest gains have been made at the 4th grade level in U.S. public schools, scores have been flat for 8th and 12th grade compared to the previous NAEP survey in 2002.

Fortunately, the picture is improving in the Cambridge, Massachusetts Public School District, says Melanie Barron, the system’s K-12 science coordinator, and a long-time advisor for Whitehead Institute’s public outreach programs.

Barron has helped to replace the lecture-dominated curriculum with more hands-on “inquiry-based” approaches, including innovative programs in epidemiology, ecology, environmental science and marine biology. Science writer Eric S. Brown recently spoke with Barron about new strategies for improving K-12 science education and how they might be expanded nationwide.

How has biology education changed in recent years?

Thanks to National Science Foundation support, the Cambridge K-8 schools now have excellent science curriculum. We have implemented a new hands-on, inquiry-based biology curriculum from Biological Science Curriculum Study called Biology: A Human Approach. In high school, we now teach physics first, then chemistry, then biology, rather than the reverse. The complexity of biology demands an understanding of physics and chemistry. The change is happening all around the country, but there’s still some skepticism out there. We need to get the curriculum to better support the shift.

Is there much skepticism about inquiry-based teaching as well?

Nationwide, there’s resistance among high school teachers. There’s a misunderstanding that if you use an inquiry-based approach, you won’t learn enough science. Yet science is not only studying the “it”—it’s doing the “it.” One of Cambridge’s high school teachers is in the Arctic on an Earthwatch expedition with four students looking at climate change. If schools were more flexible and incorporated more field-based research into the curriculum, you’d see a tremendous difference.

We need to apply instructional techniques that we’re using in elementary school to high school. High school classrooms can learn from the organization of a kindergarten room, in which you have some students working together around computers while others are reading and others are building something, and others are having a session with the teacher.

With an inquiry-based approach, will the students still be prepared for college entrance exams?

The colleges are looking for high Advanced Placement scores, but then they are complaining that the students don’t have inquiry-based, problem-solving skills. I think you can have both—they’re not mutually exclusive.

Some MIT undergraduates who volunteered in our classrooms argued that if they had grown up learning with an inquiry-based approach, they would never have gotten the scores they needed to get into MIT. Yet once these kids get into places like MIT, they revert to that inquiry-based approach, that hysterical, build-your-own-robot, steal-the-cannon style, and they do beautiful work.

What’s going on in biotechnology requires that you know how to question, how to think, how to solve problems. [Cambridge biotech firm] Microbia isn’t doing microbial research anymore because the company found a more successful direction. But if they didn’t know how to question, could they have turned the company around?

What can be done to recruit and retain qualified science teachers?

Science teachers should have regular sabbaticals to do research and visit research sites. There should be opportunities like the Whitehead Institute’s seminar series for high school science teachers. The talks are given about current research by the best researchers, and the teachers are treated like professionals.

What other challenges do biology teachers face?

The big issue is testing, testing, testing. We have a wonderful science curriculum in Cambridge, but by the 5th and 6th grades, instructional time starts to decline because teachers are told to prepare the students for math and reading tests. Above all, the teachers need to have the time to teach science.
Hwai-Loong Kong came to MIT’s Sloan School in 2001 with a mission: understanding how successful biomedical research organizations are built. The Singapore cancer physician chose Whitehead as his model for his master’s thesis.

Then he returned to his tiny Southeast Asian country to create world-class basic biomedical research institutions, serving as executive director of a new BioMedical Research Council that spearheaded the effort.

An island country just north of the equator, Singapore has a population of four million in an area that would fit within Boston’s Route 128 beltway. It has maintained impressive economic growth since the 1960s by concentrating government and society resources on targeted industries.

In the 1990s, Singapore added biomedicine to the list.

“Singapore placed a large bet on the very great potential importance and economic impact of biomedical research,” says Whitehead Member Susan Lindquist, an advisor to the country’s BioMedical Research Council during its startup years. “I think this is visionary, and has the potential to profoundly influence their future success.”

One cornerstone of the effort is the Biopolis, a new campus of government research institutes, medical schools and private research groups focused on basic biomedicine. The new facilities have attracted talent from around the world. Whitehead Member Robert Weinberg, who chairs the scientific advisory board of the Genome Institute of Singapore, remarks, “I’ve been astounded and gratified by how well they have recruited first-class researchers.”

**TRAINING GLOBALLY**

To create a new labor force to meet its biotech goals, Singapore also invested in biomedical education.

“You have to realize that this is a managed economy,” says Whitehead Member Paul Matsudaira, who has been advising Singapore on research programs in biomedicine since 1999. “In the U.S. we let a lot of people do terrific science, and then we have the confidence that it will all percolate up into useful technology, which it does. But Singapore feels it has to manage that process a little bit more efficiently because they are depending on biotechnology to support their economy. And because they want to manage their economy, they have to manage the manpower and research.”

Singapore increased opportunities for students by launching partnerships with elite educational institutions, such as MIT and Duke University. The Singapore-MIT Alliance, for example, gives students access to faculty members on both sides of the globe.

For instance, Huangming Xie, a Singapore-MIT Alliance graduate student, has two supervisors for his studies on how microRNAs regulate cell differentiation. One is Whitehead Member Harvey Lodish, in whose lab he’s working. The other, Bing Lim, is a Harvard Medical School professor who works at the Genome Institute of Singapore.

Xie is a member of the first group of doctoral students in a new program on computation and systems biology co-chaired by Matsudaira and Professor Hew Choy Leong. The group began its studies in Singapore in July 2005.

In addition to courses and research in Singapore, students benefited from videoconferenced classes taught jointly by MIT and Singapore faculty. Given the 12-hour time difference, timing was a big problem, with classes running from 8 to 10 a.m. and p.m. at the two locations, says Xie. “But the course was so interesting that we never fell asleep.”

He and his peers continued their coursework and research after arriving at MIT in January 2006. “The faculty members here are quite friendly, there’s lots of guidance and the people in the lab are very helpful,” he says. “This program is trying to link the two parts of the world together.”

Alyssa Kneller contributed to this story.
You've said recently that current funding trends at the NIH are counter-productive. Why is that?

In order to understand why this is so, we need to be clear about how scientific progress works. If you look at the history of research advances over the past century, it's clear that the dynamism of discovery research comes from small, agile research groups run by relatively young investigators who can move quickly in innovative directions in order to push forward the envelope of our understanding.

At present, however, the ability to support these groups has been undercut badly by a decrease in available funding for small, investigator-initiated research projects. Instead, more and more federal research money is going into funding large, collaborative projects.

Why is that bad?

There are two reasons.

The first, ironically, is that it indicates a misunderstanding of how scientific collaboration normally operates.

Scientists collaborate opportunistically. I'll collaborate with someone at, say, the Dana-Farber Cancer Institute for as long as the project demands. If, after a year, it's become clear that the synergies deriving from this collaboration have run their course, I'll move on and collaborate with other groups that might offer their talents in yet other areas. The key here is that I have the freedom to move, at the drop of a dime, in whatever direction the research dictates.

But these large collaborations tether scientists together for years. What do you do when you realize after a year that a collaboration is no longer useful but you still have four more years to go on the grant?

The second reason is that these types of projects naturally favor older researchers. No one at the NIH will give a brand new principal investigator $100 million to head up a project. They entrust these projects to much more established researchers. We see from the data that the age at which researchers receive their first NIH grants as independent investigators has been increasing progressively.

Between 1970 and 1980, the average age of a PhD receiving his or her first R01 award (the NIH’s basic investigator-initiated grant) was 35. Between 1995 and 2004, the average age rose to 41.

Telling a young graduate student that they'll have to wait until middle age before they can launch their career is hardly an incentive.

Can this trend be reversed?

That depends on how NIH is governed in the years to come. Those who run this large institution may come to realize that there is no greater way to continue the vitality of scientific progress than by investing in the careers of young researchers. Or a failure to change the current funding patterns will lead to a progressive decline in the quality of our biomedical research. We will continue to believe that we’re doing well, but the reality will be otherwise.
The amazing molecular motor

In terms of efficiency, car engines can’t hold a candle to a tiny motor inside your cells. The protein that cranks out ATP molecules, which power biochemical processes, wastes less than 1 percent of the energy it takes in. In comparison, car engines typically waste 75 to 85 percent of the energy stored in gasoline. Whitehead Fellow Paul Wiggins explores this incredible protein in “Under the hood: A beginner’s guide to the molecular motor,” a scientific talk without the jargon. To view the lecture, visit www.whitehead.mit.edu/news/video_gallery.

Paradigm online

Yes, your favorite institutional research magazine now gains added attractions on the Web. The online edition includes special features, such as movies. This month’s cover story on bioimaging, for example, includes videos of cells sliding across a gooey matrix or dividing to form the cavities of a brain. Visit www.whitehead.mit.edu/news/paradigm.

Listen in on protein folding

Protein folding can have profound and unexpected influences in fields as wide-ranging as human disease, evolution and nanotechnology. Whitehead Member Susan Lindquist outlines these connections in TwiT.tv’s “Futures in Biotech” podcast, available at www.whitehead.mit.edu/research/faculty/lindquist.html.

Ask a scientist

Ever wish you could grill a scientist about the latest biomedical discoveries? Here’s your chance. Email your questions to webmaster@wi.mit.edu and we’ll post them with brief responses from researchers at www.whitehead.mit.edu/programs.
CellProfiler software developed by Whitehead post-doctoral fellow Anne Carpenter avoids the tedium of looking at tens of thousands of images by eye. The software examines the original image of cells (center) until its algorithms identify the various components of each cell, such as the blue nuclei (left) and the cell edges (right). Once the cells are found, the software measures hundreds of features for every cell, such as size, shape, brightness of marked components and smoothness. See page 18 for more.