The grand challenge

What we don’t know about human embryonic stem cells
The great circle

Kicking off his first lesson for the MIT Introductory Biology course this year, Whitehead Member Robert Weinberg looked up at a lecture hall filled with more than 400 students. When he himself took the course, he told them, “there were 25 people in the class.”

But the amazing onslaught of biology discoveries is attracting disciples in droves. The life sciences have entered the mainstream in ways no one could have predicted, and the changes are only accelerating.

Unlike most MIT courses, Intro Biology is in a constant state of flux. “Calculus doesn’t change a lot year to year; most of the core work was done about 400 years ago,” said Eric Lander, co-teacher of the introductory course, director of the Broad Institute and Whitehead Member. In contrast, biologists and their collaborators are making new discoveries way too quickly for textbooks to keep up. As he told the Intro Biology students, “many of the key problems are alive and kicking—kicking a little too much.”

Every MIT student is feeling that kick. You can’t graduate from MIT now without studying biology and gaining some understanding of the latest advances.

And that understanding is seeping ever further into the mainstream, as demonstrated this spring when the Greater Boston Chamber of Commerce put its muscle behind legislation safeguarding human embryonic stem cell research in the commonwealth.

“This generation is bearing witness to a fascinating convergence of engineering and the life sciences,” says MIT president Susan Hockfield. “Together with the work of colleagues at the Broad and Whitehead Institutes, we are already seeing a torrent of new collaborations, insights and results emerging from the labs at the intersection of Vassar and Main Streets in Kendall Square.”

That crossroads is the center of what Hockfield calls “a great circle of life sciences.” No fewer than 150 biotech firms, medical-device companies and pharmaceuticals are within walking distance.

On one corner of Vassar and Main, the MIT Brain and Cognitive Science Project building will formally open this fall. Across the street is the Stata Center—a model of architectural playfulness that began housing MIT computer, information and intelligence sciences groups last year. (A number of Stata researchers collaborate with Whitehead researchers, led by Affiliate Member David Gifford.) Across the intersection are the Novartis Institutes for Biomedical Research, another frequent Whitehead partner. Broad Institute’s fancy new digs will open next year a stone’s throw away down Main Street.

And yes, Whitehead sits in the exact center of this great circle.

Eric Bender
Acting director, Communications and Public Affairs

Correction: The “Knockout Punch” article in Spring Paradigm did not accurately characterize Sirna Therapeutics’s Sirna-027. This chemically optimized short interfering RNA has shown promising early results for treating age-related macular degeneration in a Phase 1 clinical trial. We regret the error.
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MicroRNA research advances, and a blast from the Institute’s past
The human and the chimpanzee Y chromosomes went their separate ways approximately six million years ago. Ever since this evolutionary parting, these two chromosomes have experienced quite different fates.

While the human Y has maintained its count of roughly 27 genes and gene families over those millions of years, some of these same genes on the chimp Y have mutated and gradually become inactive. Whitehead researchers speculate that one likely reason for such disparity is that, compared to humans, chimpanzees have mating habits that are highly promiscuous.

“Contrary to the dire predictions that have become popular over the last decade, the sky is not falling on the Y,” says Whitehead Interim Director David Page, senior author on a study that appeared this September in Nature.

“This research clearly demonstrates that natural selection has effectively preserved regions of the Y chromosome that have no mechanisms with which to repair damaged genes,” he says.

For many years, it’s been assumed that the Y chromosome is headed for extinction because, unlike other chromosomes, it has no genetic “mate” with which to swap genes.

But in 2003, Page published a landmark paper in Nature challenging that claim by demonstrating that a certain region of the Y chromosome possessed a unique mechanism for repairing mutated genes.

Through sequencing the Y, the Page lab and collaborators at Washington University School of Medicine in St. Louis discovered that many of its genes were located in palindromes—long stretches of DNA letters that read the same forwards and backwards. By folding into a hairpin, the authors suggested, a gene might then swap the appropriate genetic material with itself. In this way, the Y chromosome could maintain its integrity despite lacking a mate.

However, there is another region of the Y, called the “X-degenerate” region, where the genes are not situated in palindromes.

“The genes in the palindrome region are primarily sperm-producing genes, and most other genes unique to the Y aren’t located there,” says Jennifer Hughes, a postdoctoral scientist in Page’s lab and first author on the paper. These other genes have no obvious means for self-repair. Because of this, many proponents of the “Y’s demise” theory remained undaunted.

Once again collaborating with Richard Wilson from the Washington University School of Medicine in St. Louis, Missouri, Page and his research team sequenced this X-degenerate region of the chimpanzee Y chromosome and compared it to the human Y.

“We were looking for any evidence that the human Y has lost genes since parting ways with the chimp,” says Hughes. “Had we found active genes on the chimp Y that had become inactive on the human, that would be the smoking gun. But we didn’t find any such evidence. In fact, we found the opposite.”

On the chimp Y, five genes have suffered mutations that rendered them inactive. On the human Y, those same genes continue to function perfectly. “Even though the Y has lost many genes since its origin
about 300 million years ago,” says Hughes, “it’s been holding steady in humans for the last six million years.”

In other words, if the one region of the Y can depend on itself for survival, the other region has found a friend in evolution.

“We now see that natural selection is working to conserve this unpartnered region of the Y,” says Page. “If mutations do occur in any of these genes, they don’t seem to pass on in the lineage. This is a clear example of how evolution is not just about moving ahead, it’s also about not falling behind.”

Male chimps, just like male humans, are probably not bound for early extinction. Those genes in the X-degenerate region are what scientists call “housekeeping” genes—active in most cells in the body and not carrying out male-specific functions.

Page and his team speculate that the loss of genes on the chimpanzee Y may be due to the chimp’s mating habits. Both male and female chimps engage with multiple partners when they mate. This puts a strong selective pressure on those genes that produce sperm. Conversely, it puts less pressure on evolution to preserve those genes on the Y whose functions have nothing to do with reproduction. Because humans historically have been largely monogamous, our Y chromosomes have been spared such selective-pressure imbalance.

“Of course,” acknowledges Page, “this is a hypothesis that we have no way to scientifically prove or disprove. However, we believe it’s currently the best explanation.”

Why is melanoma so malignant?
Deadly skin cancer reawakens dormant cellular process

About 60,000 Americans will be diagnosed with melanoma this year, says the American Cancer Society, and 10,000 of those cases will be fatal. If not caught in the early stages, melanoma can be a particularly virulent form of cancer, spreading with an efficiency that few tumors possess. Now, Whitehead researchers have discovered one reason why this skin tumor is so ruthless. Unlike other cancers, melanoma is born with its metastatic engines fully revved.

“Other cancers need to learn how to spread, but not melanoma,” says Whitehead Member Robert Weinberg, senior author of a paper published in the journal *Nature Genetics*. “Now, for the first time, we understand the genetic mechanism responsible for this.”

Metastasis (the spread of disease to an unconnected body part) is a cumbersome, multi-step process that demands much of the cancer cells. Researchers have wondered why melanoma is able to do this more efficiently and at a far earlier stage than other cancers. This new study shows that as melanocytes—cells that produce pigmentation—morph into cancer cells, they reawaken a dormant cellular process that lets them travel swiftly throughout the body.

Central to this process is a gene called Slug, named after the bizarre embryo shape that its mutated form can cause in fruit flies (see “Twist of Fate,” *Paradigm*, Fall 2004). Slug is active in the neural crest, an early embryonic cluster of cells that forms a variety of cell types in the adult, including melanocytes. In this early embryonic stage, Slug enables the neural crest cells to travel, and then settle, throughout the developing embryo.

“Slug is a key component of the neural crest’s ability to migrate,” says Piyush Gupta, a graduate student in Weinberg’s lab and first author on the paper. Eventually it shuts off in adult tissues. But when skin cells become malignant, they readily reactivate Slug and gain the ability to spread—something that other cancers can spend decades trying to do.

Weinberg’s team demonstrated this through a number of experiments. First, they injected cancer cells underneath the skin of mice and found that while other types of cancers formed only localized tumors, the melanoma cells immediately created tumors throughout the body from the lungs to the spleen. For the second experiment, the team used microarrays and found that Slug was expressed in human melanoma. Finally, the research team found that when Slug was knocked out in melanoma cells, the cancer didn’t metastasize when placed into a mouse.

“This work is yet another demonstration of the notion that certain embryonic genes normally involved in transferring cells from one part of the body to another are also involved in enabling cancer cells to spread,” says Weinberg.
As medical procedures that compromise a person’s immune system become more common, opportunistic infections that exploit these weaknesses are an increasing public health concern. Pathogenic fungi are chief among these invaders that prey upon organ-transplant recipients and chemotherapy patients—and they have demonstrated an alarming ability to quickly sidestep drug treatment.

Now, researchers in the laboratory of Whitehead Member Susan Lindquist have identified a mechanism that enables pathogenic fungi to evolve drug-resistant capabilities with such distressing rapidity.

“These findings have broad therapeutic implications for managing life-threatening fungal infections,” says Lindquist, whose paper appeared in September in the journal Science. “We also now have a clearer understanding of the forces that shape a new trait and the molecular mechanisms by which it is accomplished. The potential to evolve is an inherent biological property of the organism.”

“The emergence of drug resistance in pathogenic microbes provides a resounding validation of Darwinian evolution,” Joseph Heitman of Duke University Medical Center commented in a perspective piece that accompanied the paper.

For many years, Lindquist and her colleagues have been poring over a protein called Hsp90 (see “Within the folds, outside the box,” page 13). Hsp is an acronym for “heat shock protein,” meaning that the protein responds rapidly to certain environmental stresses, such as elevated temperatures.

But Hsp90 is also a “chaperone protein,” a member of a family of proteins dedicated to helping fellow proteins assume their proper shapes. Proteins fold into a vast array of conformations whose precision is essential to the cell’s well being. One misfold can prove toxic to the cell. Hsp90 directs this folding process in a wide range of crucial proteins.

In a 2002 Nature paper, Lindquist and her research team found that by lowering levels of Hsp90 in plants, they could cause sudden and unexpected changes in a vast array of traits. (These results paralleled a 1998 Nature paper in which Lindquist and colleague Suzanne Rutherford discovered the same mechanism in fruit flies.) As a result of these findings, the team deduced that these plants must gradually accumulate genetic variations that Hsp90 somehow manages to keep in check. But if Hsp90 is compromised, perhaps due to an environmental stress, this reserve of dormant mutations is suddenly unleashed.

Leah Cowen, a postdoctoral scientist in the Lindquist lab, was interested in using the model yeast Saccharomyces cerevisiae to investigate how Hsp90 might contribute to evolution. She also wanted to study whether Hsp90 enabled pathogenic fungi like Candida albicans and Aspergillus species to resist treatment.

Cowen designed experiments in which fungal cells were exposed to two common classes of antifungal drugs: azoles and echinocandins. She genetically engineered fungal strains to have either high levels of Hsp90, or low levels, and then exposed them to these drugs. Results were striking.

“Strains with high levels of Hsp90 could rapidly evolve drug resistance, while the ability of strains with low levels of Hsp90 to evolve resistance was impaired,” says Cowen. She also looked at strains that had evolved drug resistance through mechanisms other than Hsp90. Even in these cases, “when Hsp90 levels were reduced these new resistance traits were lost.”

Additionally, Cowen found that once fungal strains became drug resistant, they could eventually evolve the ability to retain this resistance independent of Hsp90.

“Ultimately,” says Cowen, “these results establish a new role for Hsp90 in the evolution of adaptive traits.”

“We are extremely excited about the potential of Hsp90 inhibitors for treating infections in the clinic,” adds Lindquist. Drugs that inhibit Hsp90, at least in theory, could both render these pathogens more responsive to treatment and prevent them from ever developing such resistance in the first place.
Whitehead researchers have discovered another possible reason why pathogenic fungi are such a scourge. According to the research published in *Nature Genetics*, fungal microbes can quickly alter the appearance of their cell surfaces, their “skin,” disguising themselves in order to slip past the immune system’s vigilant defenses.

“It’s all about skin,” says Whitehead Member Gerald Fink, who compares the fungal microbe to an M&M—a sugar coating encasing the cell’s DNA. “The skin of fungi microbes is what enables them to stick to your organs, and thus become pathogenic.”

The genetic core to this study is a DNA phenomenon known as tandem repeats. Here, small units of between 3 and 200 nucleotides form within a gene and repeat sometimes up to about 35 times.

Kevin Verstrepen, a postdoctoral researcher in Fink’s lab, scanned the entire yeast genome and discovered that these repeats are not only common, but that more than 60 percent occur in genes that code for cell-surface, or skin, proteins. “Most of these repeats somehow affect how the yeast cell interacts with the environment surrounding it,” says Verstrepen.

In addition, he found that the length of these repeats varied greatly between a mother and a daughter cell. A cell can lose a 20-unit repeat with one cell division, then immediately gain it back with the next division.

This provides a significant clue into why fungal infections can be so deadly. If these fungal microbes can quickly reconfigure their surfaces by changing the number of repeats in a gene, they can then manage to stay one step ahead of our body’s defenses.

“These microbes have been around for billions of years,” says Fink. “They haven’t come this far without learning how to fight off predators.”

Verstrepen and his colleagues followed up with study of a gene called FLO1, common to both baker’s yeast and pathogenic fungi. FLO1 creates the conditions that enable yeast cells to glom onto to surfaces and pathogenic fungi to stick to host tissue. The researchers discovered a clear correlation between the number of repeats in FLO1 and the degree to which these cells could attach to a surface. When FLO1 contained many repeats, it adhered vigorously to a plastic surface. As the number of repeats declined, so did its ability to adhere.

Fink believes that these findings show why traditional approaches to targeting drugs won’t work. “We need to target other aspects to the cell surface that don’t change,” he says.
New tools for an old can of worms

TODAY’S HIGH-POWERED ANALYSES BEGIN TO ANSWER THE QUESTION OF HOW FLATWORMS REGROW

BY ERIC BENDER  Illustration by Stuart Bradford

This lab exercise is simple—although not necessarily for the squeamish.

Take one live planarian flatworm. Chill it nicely in an iced Petri dish. Using a tiny microsurgical blade, cut the centimeter-long beast into as many pieces as you can stand, and plop them in a plastic bowl. Store it in the dark.

After a week, you’ll see fully formed planarians swimming around in your bowl.

For many decades, scientists have been carrying out versions of this experiment and banging their heads against the wall trying to understand the result. It turns out that you can cut off a planarian slice as small as 1/279th of the animal and have it turn into a complete adult. (That finding, by the way, comes from Thomas Hunt Morgan, who gave up on planarian regeneration studies in frustration and turned to his pioneering work in the genetics of fruit flies.)

For many centuries, inquiring minds have puzzled over the capabilities of certain worms, amphibians, fish and other animals to regrow limbs and other body parts. Serious scientific inquiry goes all the way back to 1740, when Abraham Trembley experimented with hydra. But while researchers carefully documented, for example, exactly what happens if you cut planarians into quarters, the underlying mechanisms remained entirely mysterious. And while developmental biology has exploded in recent decades, the field of regeneration biology remains rather small.

“Regeneration is one of the great mysteries of biology that has puzzled developmental biologists for well over a century,” says Whitehead Associate Member Peter Reddien. But that’s changing quickly as researchers bring the powerhouses of modern biological analyses to studying these processes—with the hope that a better understanding of regeneration may eventually find medical applications.
Planarians such as *Schmidtea mediterranea* live in freshwater streams and ponds, eating insects and avoiding light, which they spot with the simple photodetectors that give them a cross-eyed look. Their digestive and nervous systems appear rudimentary.

But how they reproduce is not rudimentary at all. Some strains reproduce as cross-fertilizing hermaphrodites (both worms containing both sperm and eggs and simultaneously fertilizing each other). Other strains reproduce by dividing in half, with both head and tail forming a new animal much like the amputated chunks.

New planarian tissues and organs are created by neoblasts—adult stem cells that share certain characteristics with embryonic stem cells and can differentiate into essentially all the cells in adult animals. This process occurs in both chopped-up and normal worms. Just how it works is still almost completely unknown.

Biology’s major model organisms aren’t much help either, declares Alejandro Sánchez Alvarado, Howard Hughes Medical Institute investigator and professor of neurobiology and anatomy at the University of Utah. (Reddien worked as a postdoc in Sánchez Alvarado’s lab before arriving at Whitehead this year.)

The *C. elegans* worm and the *Drosophila* fruit fly, “the warhorses of developmental biology,” flunk out on this test. “Pull a wing off of a *Drosophila*, and it won’t grow back,” says Sánchez Alvarado. “Take a *C. elegans* and slice it in half, and it will die.”

Sánchez Alvarado has been working since the late 1990s to turn *S. mediterranea* into a model organism. He chose the worm for its regeneration capabilities, developmental plasticity, ability to reproduce both sexually and asexually, and its unusual stem cells. It didn’t hurt that *S. mediterranea* is relatively easy to work with in the lab, and that it later turned out to perform functions with relatively few genes per function. He and his coworkers have been steadily accumulating knowledge through new laboratory tools.

**RNAi and the regeneration gap**

Most dramatically, they’ve employed the use of RNA interference (RNAi), a technique that can knock out the function of individual genes.

Reddien led the first high-throughput RNAi screen of planarian genes during regeneration, with results published this May in *Developmental Cell*. The researchers painstakingly silenced 1,065 genes one at a time, and found that 240 of these genes, when silenced, produced defects in the worms.

No fewer than 204 had corresponding genes in other species. “There’s a large degree of conservation between the genes that are affecting regeneration efficiency in planarians and genes in *C. elegans*, *Drosophila* and humans,” says Sánchez Alvarado. That’s intriguing because highly diverse organisms often develop via very similar molecular pathways. “Neurons and muscle cells, say, are all made much the same way by hydras and humans,” he points out.

While 145 of the silenced genes were essential for regeneration and homeostasis (normal tissue loss and replacement), other genes were required for one or the other but not both. This suggests separate molecular pathways for homeostasis and regeneration—an encouraging sign for regeneration studies.

The team also found a wealth of leads for further research, including a novel gene apparently involved in wound healing.

At Whitehead, Reddien is plunging ahead with additional RNAi screening, which “works incredibly well for planaria,” he says. He and his colleagues have found they can express double-stranded RNAs in bacteria, and introduce the bacteria into the planarian’s liver-and-agar lunches. The double-stranded RNA spreads through all cell types and shuts off the targeted gene.

The *S. mediterranea* genome is being sequenced at the Genome Sequencing Center at Washington University in St. Louis. Sánchez Alvarado, Reddien and Philip Newmark wrote the white paper that brought funding from the National Human Genome Research Institute.

For now, though, “we are still amazingly ignorant about the cell biology of what’s happening in regeneration,” cautions Newmark, an assistant professor of cell and structural biology at the University of Illinois in Urbana.

“Regeneration is in the same state as developmental biology was at the start of the 20th century,” as Sánchez Alvarado puts it. With scientists just starting to unveil the molecular frameworks for cellular processes, “we have no clear pictures of what the stem cells are doing throughout the day,” he says.
A colony of human embryonic stem cells from a presidentially approved line. Such cells occur naturally in embryos a few days old, in a hollow microscopic ball of cells called a blastocyst.

What we don’t know about human embryonic stem cells could fill labs all around the world.

By Erika Jonietz

The Grand Challenge

First came the egg—in this case, an ordinary mouse egg.

Scientists removed the egg’s nucleus and replaced it with the nucleus from a skin cell of a mouse suffering a genetic immune deficiency. Next they manipulated the egg to develop into a blastocyst, a hollow ball holding the embryonic stem cells with the potential to become any cell in the body. The researchers then plucked out the stem cells, corrected the genetic defect, and used the cells to treat the immune deficiency. And the mouse was partially cured.

Announced three years ago by the labs of Whitehead Member Rudolf Jaenisch and then Whitehead Fellow George Daley, this was the first successful “proof of principle” that somatic cell nuclear transfer actually could help to cure disease.

But this fall as Jaenisch opens the Whitehead Human Embryonic Stem Cell Facility, he won’t be working directly toward replicating this achievement in humans.

Instead, the first order of business is to study the cells’ basic biology. Deep and tough problems must be solved long before embryonic stem cells can become useful clinically, Jaenisch says.
GROWING PAINS

One obstacle is simply in learning how to work with these enigmatic cells.

In 1998, James Thomson at the University of Wisconsin-Madison launched the field by deriving the first human embryonic stem cells using embryos from in vitro fertilization clinics. Despite seven years of experience and the creation of more than 100 stem cell lines worldwide, scientists still do not know the best methods for deriving and growing them.

Biologists have encountered severe problems growing the earliest stem cell lines, including most of the lines eligible for federal research funding under President Bush’s 2001 decree. “Those lines are very hard to grow and very hard to keep pristine,” says Irving Weissman, director of the Stanford Institute for Stem Cell Biology and Regenerative Medicine. He notes that many of the first lines derived now show genetic abnormalities that limit their utility.

“We are far from knowing what the optimal conditions are for culturing human embryonic stem cells,” agrees Kevin Eggan, assistant professor of molecular and cellular biology at Harvard University. “So when we derive them and culture them, we are almost certainly doing things that mess them up.”

WHAT MAKES A STEM CELL?

Even as they struggle to grow human embryonic stem cells, biologists also face basic questions about how they work. The most fundamental of these is “stemness”—what makes a stem cell a stem cell.

Scientists are just beginning to work out the internal programs and external cues that give the cells their unique ability to become any other type of cell, that maintain them in this state, and that allow them to self-renew, almost indefinitely.

Whitehead Member Richard Young, collaborating with Douglas Melton, co-director of the Harvard Stem Cell Institute, is mapping the internal mechanisms and gene/protein interactions involved in stemness.

To uncover the proteins that control gene expression in stem cells, the team is employing techniques that Young helped develop to study the regulatory pathways of baker’s yeast. “There is a perception that human embryonic stem cells may be useful for regenerative medicine,” Young says. “But before we get there, many of us believe that we have to understand these pathways a little better.”

Like Young, Princeton’s Ihor Lemischka is tapping systems biology techniques, such as gene chips that represent the total genome, to identify all the genes that are active in embryonic stem cells but not in more mature cells. Austin Smith, director of the Institute for Stem Cell Research at the University of Edinburgh, has taken more classical genetic and biochemical approaches to working out the molecular pathways for self-renewal, creating mutations in specific genes or exposing cells to different substances to test their effects.

GETTING WITH THE REPROGRAM

Understanding these pathways will help researchers with another...
quest of stem cell biology: deciphering how transferring the nucleus of an adult cell into an egg effectively reprograms that nucleus, resetting its genes to the beginning of embryonic development.

Scientists have employed this technique to clone animals, starting with Dolly the sheep in 1997. In 2002, Hwang Woo Suk and colleagues at Seoul National University used it to derive a stem cell line that matched a specific patient. “Understanding reprogramming at a basic level could have a major impact on the whole field,” says Leonard Zon, professor of pediatrics at Harvard Medical School and past president of the International Society for Stem Cell Research.

Knowing how the process works could reward stem cell scientists doubly. It would give them a way to derive stem cells without using embryos (thus avoiding that ethical controversy). It also would provide a means of cloning stem cells customized to treat individual patients without the need for more egg cells.

“For everyone’s idealized vision of personal cell replacement therapy to come true, we will need something like this,” says Eggan.

Eggan gained great proficiency with nuclear transplantation technology as a graduate student in Jaenisch’s lab. In his Harvard lab, he investigates whether embryonic stem cells might contain the same unknown substances that stimulate reprogramming in egg cells.

### DECODING GENETIC DISEASE

Even in its current state, nuclear transfer technology offers biologists a unique tool for studying genetic disease. Most dramatically, in June Hwang and his colleagues used it to create embryonic stem cell lines from patients with type 1 diabetes and a genetic immune deficiency.

The technique, Weissman says, will enable researchers to figure out exactly how such diseases develop—discoveries they may not be able to make any other way. “That’s why it’s such a big platform technology,” he says. “You can make a cell line with a genetic disease, you can study it in a test tube, you can send it around to everybody who’s interested, and you can also put it into an animal model where there’s a chance that the disease will happen.” Weissman hopes to eventually produce such stem cell lines at Stanford with backing from California’s Institute for Regenerative Medicine.

Eggan has requested permission from Harvard to derive human embryonic stem cell lines from patients with Parkinson’s and Lou Gehrig’s diseases using private funding.

### TRAINING TISSUES

Biologists have been trying to create particular cells and tissues from human embryonic stem cells since their discovery. Without an understanding of underlying developmental pathways, progress has been slow.

The Oct4 protein, shown in green, plays an essential role in controlling the ability of embryonic stem cells to differentiate into other cells. These are cells from the presidentially approved HSF-6 line, developed at University of California, San Francisco.

**2002**
- Bongso and colleagues establish a human embryonic stem cell line without using animal cells

**2004**
- Hwang Woo Suk and colleagues at Seoul National University clone a stem cell line from a human embryo
- The United Kingdom Stem Cell Bank launches, with two embryonic stem cell lines

**2005**
- Seoul team clones stem cell lines from adults, including a patient with type 1 diabetes
- Whitehead’s Young lab and partners offer early snapshot of regulatory framework for human embryonic stem cells
Four big questions

Here’s a sampling of the human embryonic stem cell issues being tackled by Whitehead scientists

1. **What makes an embryonic stem cell a stem cell?** Using microarray technology developed at the Institute, Whitehead Member Richard Young, collaborating with Rudolf Jaenisch and others, has discovered the first layer of circuitry that enables such cells to be pluripotent, suppressing entire networks of genes essential for later development.

2. **How can we manipulate embryonic stem cells?** Jaenisch and Young are exploring ways to influence key genes and proteins, as well as tampering with their regulatory circuitry. Understanding these processes is a prerequisite for systematically coaxing embryonic stem cells into forming particular cell types.

3. **How does an egg reprogram the genome in somatic cell nuclear transfer?** The Jaenisch lab is investigating the exact biochemical processes that the egg uses to reactivate the donor nucleus during somatic cell nuclear transfer. The long-term goal is to turn a mature cell into an embryonic stem cell without requiring an egg.

4. **How can we make adult stem cells out of embryonic stem cells?** In this area, embryonic stem cell researchers and adult stem cell researchers need to work together. The Lodish lab is working to create adult blood cells out of embryonic stem cells, drawing on the expertise of Jaenisch, Young and Whitehead Fellow Fernando Camargo.

Kevin Egger’s group successfully fused an embryonic stem cell with an adult skin cell.

into therapeutic hopes for embryonic stem cells. Zon and Daley, for example, want to cure diseases such as sickle cell anemia and immunodeficiency disorders.

But existing techniques for differentiating embryonic stem cells into specific cell types have proved inefficient, leading to mixtures of cells at different stages of development.

“If you read the papers, you’ll see people say it looks like three or four percent of the cells can develop into heart cells, or 80 percent seem to be motor neurons,” says John Gearhart, director of stem cell biology at the Johns Hopkins Institute for Cell Engineering. “We see these numbers all over the place, and invariably this tells you how inefficient the system is.”

**CROSSING PRESIDENTIAL LINES**

As they work to answer these key questions, each of these researchers either uses or plans to use non-presidential embryonic stem cell lines.

While the presidential lines have already helped biologists with some basic research, all of them were derived using mouse cells or components of animal blood to feed the embryonic stem cells. This not only makes the lines unsuitable for clinical use but also complicates basic science.

Researchers have recently begun deriving human embryonic stem cell lines without using any animal tissue, lines that Gearhart thinks will give a big boost to the field. “We would certainly benefit enormously by new lines that don’t require mouse feeder layers,” he says. “I can’t express how much of a pain they are.”

Without mouse feeder cells, it should be easier for scientists to investigate the mechanisms that keep stem cells undifferentiated and control their development, he adds. And when biologists ultimately succeed in producing specific cell types such as neurons or muscle cells from such animal-free embryonic stem cell lines, those cells might be moved more directly into clinical trials.

The presidential lines “aren’t sufficient,” Jaenisch declares flatly. “They are not behaving the way they should be anymore.”

So at least for now, Whitehead’s new lab must be funded entirely with private money. But private funding cannot completely fill the gap for U.S. researchers. “The National Institutes of Health are the key funding source,” says Jaenisch. “If they’re not there, it is a major, major problem.”

And even when researchers manage to obtain private backing, the federal funding ban has made it much more difficult for those wishing to work with non-approved lines (see “Laying down the laws for stem cell research,” page 22).

As they wait for public opinion and political will to match their need, U.S. researchers work with what state and private money they can raise. As the field races ahead worldwide, Whitehead scientists aim to stay in the vanguard. “Embryonic stem cells hold enormous potential,” says Jaenisch. “We have to be sure that we can realize that potential.”
Within the folds, outside the box

Susan Lindquist uncovers the roles that misshapen proteins play across an astonishing sweep of phenomena

BY CAROL CRUZAN MORTON
The challenges to these different critters have been limited only by the overactive imaginations of Lindquist and her colleagues. One recent episode involved forcing yeast cells to evolve resistance to the best drugs used in clinics to fight deadly fungal infections. In future installments, the yeast must first imitate and then overcome a protein problem underlying a horrific human neurodegenerative disease.

The amazing but true stories of how some of the yeast have endured, and even thrived, have advanced a strangely wide array of science: mad cow disease, neuroscience, nanotechnology, cancer, anti-fungal drug resistance and non-genetic evolution.

But these seemingly disparate discoveries share a common thread. “The one universal theme in our lab is protein folding and how changes in protein folding drive many biological processes,” explains Lindquist in her sixth-floor office overlooking MIT on a hazy day. “People didn’t realize how broad the protein folding problems are. A lot of things that started out as basic research into protein folding are now translating into a direct interest in human health and medicine.”

Researchers in her lab have even found that proteins can trump DNA in passing along new traits into the genomes of future generations. “You don’t usually think about proteins this way,” acknowledges James Shorter, a senior research associate. “Independent of the underlying DNA, protein folding can influence a wide variety of things, from evolution to disease progression and initiation. And it can act as a genetic element. Initially, this seemed just crazy, but it is true.”

“A distorted protein may be unable to carry out its crucial mission, or it may have transformed into something nasty. Either way, “it can be an absolute disaster,” Lindquist says. “Misfolded proteins are responsible for many terrible illnesses of mankind. In cystic fibrosis, just one amino acid in several hundred is wrong. This means that this one protein can’t quite fold up properly to get to the surface and function. Disaster.”

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Feverishly hot climates. Dizzying alcohol and sugar binges. Heavy metals. Toxic drugs. Genetic mutations. Over the years, yeast, fruit flies, mustard plants and mice have struggled through their own versions of an extreme reality TV show in the laboratory of Whitehead Member Susan Lindquist.
Surprisingly, her lab is finding that in some circumstances, an alternative fold in a protein may underlie vital aspects of normal biology.

**A MODEL MENAGERIE**

Lindquist, who started as a fruit fly cell biologist, now tracks warped proteins and their consequences through model systems spanning millions of years of evolution.

She switched to yeast as her main model 22 years ago after attending the Cold Spring Harbor Yeast Genetics Course co-led by Whitehead Founding Member Gerald Fink. But the lab nimbly moves through an experimental menagerie that also includes plants, mice and human cancer cell lines. In collaborations with others, the team has added expertise in rats and sea slugs.

For example, in a sea slug’s oversized neurons, the protein that sustains long-term memory at the junctions and synapses seems to work by shifting its shape into a prion, a configuration that bends other proteins to its same altered form.

This finding, reported two years ago in collaboration with neurobiologist Eric Kandel at Columbia University, complemented unexpected aspects of prion activity in yeast. It contradicted a widely held belief that prion activity is inevitably toxic, a generalized assumption inspired by the well-publicized and frightening cases of mad cow disease transmitted to people.

And the slug protein work suggested an unexpected new mechanism for long-term memory in higher organisms as well. “Protein folding is deeply rooted in biology,” Lindquist says. “All organisms face the same problems and share the same solutions. Mother Nature has been coping with protein folding problems since the dawn of time. It makes sense that she would discover ways to turn it to her advantage.”

**HEAT SHOCK AND AWE**

Potentially devastating protein folding problems worsen when a cell or organism is stressed by hostile environmental factors, such as heat. In response, cells send in a rescue squad called heat shock proteins, also known as chaperones, to resuscitate or cart away proteins missing their full complement of nips and tucks.

Some of the emergency workers prevent unfolded proteins from aggregating. Some disaggregate dysfunctional clumps. Some hold proteins in partially folded states until they receive the right signal, perhaps from a hormone. Some act as trash collectors for the irredeemably malformed. Others refold wilted proteins and give them a second chance.

In a sense, heat shock proteins also have chaperoned Lindquist’s career, beginning with her independent decision to study them in fruit fly tissue culture when she was a graduate student in the Harvard lab of Matthew Meselson. Then, she was more interested in the rapid change in gene expression patterns stimulated by environmental stress, which is anything but subtle. Indeed, it is a cellular shock and awe tactic, with genes churning out 50 to 1,000 times more heat shock proteins to try to save a cell from its environment.

At the University of Chicago, where she did her postdoctoral work and progressed to full professor and Howard Hughes Medical Institute investigator, Lindquist helped unmask the function of heat shock proteins in the protein folding response.

Back in her fruit fly days, Lindquist and her collaborators had figured out that one known heat shock protein, Hsp90, worked by helping proteins with minor mutations that would otherwise alter their form. Taking away Hsp90 unveiled complete sets of hidden mutations with new functions. If the new variations were advantageous, Lindquist’s team showed, the breeding flies’ offspring hung onto the...
helpful mutations and the remodeled proteins, even without the assistance of Hsp90.

Three years ago, Lindquist’s group announced that Hsp90 performs the same trick in the experimental plant thale cress, making it likely that other organisms can also save up genetic changes for a rainy day. The mechanism could help to explain some of the rapid diversification found in the fossil record.

On a darker note, the group also has found that Hsp90 enables mutations in human cancer cells to promote cancer growth. In animals, Hsp90 inhibitors can reverse oncogenic transformations and are now in clinical trials.

Most recently, postdoctoral researcher Leah Cowen has shown that Hsp90 allows yeast to rapidly evolve resistance to antifungal drugs (see “How pathogenic fungi evolve drug resistance,” page 4). Once resistance has evolved, compromising Hsp90 functions with drugs or mutations can abolish it. Remarkably, yeast exposed to temperatures that simulated human fevers lost drug resistance, mimicking the effects of inhibiting Hsp90 function. This provides one of the first molecular explanations of a beneficial role for fevers.

**PROTEIN BUSTERS**

In yeast, Lindquist discovered another member of the heat shock family, Hsp104. This protein proved to be a powerful protein remodeling agent that saved yeast from sudden high-temperature heat shocks and all sorts of other stressful environmental conditions her lab could conjure.

And it seemed to be doing the impossible. Unlike Hsp90, which holds onto proteins and prevents them from misfolding, Hsp104 works to take apart proteins that have aggregated together. That finding reversed a common dogma that misfolded and aggregated proteins are irredeemable, Lindquist said. Instead, the chaperone rescues congealed proteins and restores them to their individual functions. “You can’t unfry an egg, but you can uncoddle an egg,” she says.

Strikingly, Hsp104 can also pass along and release hidden genetic variation. As part of normal yeast biology, Hsp104 remodels a protein named Sup35 into a prion named [PSI+]. Lindquist’s team showed that Hsp104 was necessary to refold Sup35, but once transformed, [PSI+], a regulator of protein synthesis, is positively evangelical about converting other Sup35 proteins to the same altered shape. Hsp104 ensures that mother cells pass along the prion to daughter cells, whose proteins are thereby influenced to keep changing shape, too.

This goes on for generation after generation.

Why would cells have a protein that changes shape like this? [PSI+] removes the stop sign that normally appears when proteins are being synthesized: Ribosomes roll through their normal stopping point on an RNA strand and read into fresh genetic regions. Many proteins are outfitted with extra features, which may provide a survival advantage in a fluctuating environment and thus eventually become genetically fixed.

Perplexingly, the prions created by low levels of Hsp104 can be disaggregated by high levels of Hsp104. Last year, Shorter helped resolve this major conundrum, publishing in the journal *Science*. Shorter worked out the complicated and dramatically shifting biochemistry by mixing the two proteins Hsp104 and Sup35 in a test tube with various sources of energy.

“This is the first time that anyone has found anything that can catalytically take apart an amyloid fiber,” Lindquist says. In the lab, protein amyloids, like those that clog up the brains of people who died from Alzheimer’s disease, are impervious to just about anything, including extreme heat and cold and powerful detergents.

The yeast prion amyloid fibers are also remarkably resilient, able to withstand exposure to extended high temperatures, high and low salt, strong alkalis and acids, and 100 percent ethanol.

Before coming to Whitehead, in fact, Lindquist and her collaborators at the University of Chicago exploited the strength of these protein-protein connections to make nanoscale wires of gold and silver a thousandth the thickness of a human hair that successfully conducted electricity.

In the last few billion years, animal cells lost the ability to produce Hsp104. “You can imagine how it might be useful for diseases associated with protein aggregation,” Shorter says. “If we understand how it works, we can apply it to other systems.”

**THE MAD COW CONNECTION**

The brain of a victim killed by a prion disease, such as mad cow disease, typically is clogged with clumps of the prion protein PrP that has entered a rare, misfolded state called PrPSc.

In 2002, Lindquist and Ohio State University’s Jiyan Ma suggested a unifying theory that can help explain how these devastating diseases get started and how they kill.
nothing in common. But the ability of [PSI+] to self-replicate by changing the shape of other proteins is eerily similar to the way the infectious mad cow protein seems to corrupt a plentiful membrane protein in people’s brains into an insidious shape that causes horrific disease. And the two proteins have one vaguely similar region.

Further experiments in yeast and mice along these lines led Lindquist to propose a new, unifying hypothesis to explain the origin of the human prion disease and the mechanism of its toxicity.

Bits of misfolded proteins processed by specialty organelles may accumulate in the main compartment of the cell, the cytosol, where they can be tagged for disposal by the cellular garbage service. The volume may cross a threshold, where the cell’s quality control systems cannot remove the misfits fast enough. Even a barely detectable level of misshapen proteins can be toxic to a neuron.

PROBING PARKINSON’S

Using the yeast as a living test tube, a team led by graduate student Tiago Outeiro has showed that overproduction of a human protein, alpha-synuclein, can convince neighboring proteins to abandon their normal shape and form protein clusters similar to those in Parkinson’s disease. The afflicted yeast suffer from a similar range of symptoms and die.

“We have reason to believe it is a quality control problem,” Lindquist says. “In some people, the protein misfolds at a higher rate, and that becomes a disaster in a hurry. In other people, as they age, the protein folding quality control system gets wimpy and can’t keep up with the normal rate of misfolding.”

Her team screened 116,000 chemical compounds to reverse the toxicity of alpha-synuclein overload in yeast. Among the 60 compounds, they found one that previously had been used as an antibiotic and is now in clinical trials for Alzheimer’s disease. “That makes me think we’ve found something real,” Lindquist says. “We hope we will be able to develop therapeutic strategies in yeast.”

Postdoctoral fellow Aaron Gitler now is searching for the original defect that the Parkinson’s protein triggers in yeast cells in hopes of identifying the underlying disease pathway and key drug targets.

OUT OF BOUNDARIES

Not surprisingly, Lindquist can’t predict where this rich and deep collection of studies will lead her.

“I hope it won’t be something I anticipate now,” she says. “Seventy percent of what I’m now doing I couldn’t have foreseen five years ago.

“It happens in other labs too. You take unexpected twists and turns not only from your own data but from responding to the scientific community at large.”

Whatever the future brings, Lindquist is likely to be more closely involved in human diseases. Last year, she co-founded FoldRx Pharmaceuticals, which will develop drugs to treat diseases of protein misfolding. She also was elected to the board of directors for Johnson & Johnson.

“Susan has enormous creativity,” says close friend Elaine Fuchs, a Howard Hughes investigator at Rockefeller University and a member of Whitehead’s Scientific Advisory Board. “Her ability and vision to think about areas of science so broadly allow her to make connections that are quite extraordinary and lead to interesting science.” Those significant connections extend beyond science, adds Fuchs, whose marriage resulted from Lindquist’s penchant for matchmaking.

Lindquist puts it another way. “There’s a great deal to be said for concentrating on one thing,” she says. “I’m the exact opposite.”
You’re packed into a crowded elevator when the woman beside you sneezes. Unsurprisingly, you keep breathing. As you step out of the elevator, vaporized particles from the sneeze make their way deep into your lungs and take hold. And so the war begins.

It’s a war your body is always fighting. Microbes are everywhere, and some are out to get you. Fortunately, you’re armed with multiple layers of defense, starting with your skin. But some of those microbes will find a way in. And that’s when they confront your immune system.

Think of the human immune system as your own army of defense, an integrated force of tissues, organs, cells and molecules that seek and destroy any cell whose membrane—or skin—looks fishy.

But there’s nothing simple about this war. The enemy fights back, with tricks based on the selective pressures of Darwinian evolution. Often a few microbes with some sort of genetic variant or mutation escape detection. These gain a survival advantage and begin replicating. Now the immune system must learn to destroy these new, mutated microbes. “The very fact that you have a defense mechanism against these pathogens puts a selective pressure on them to bypass it,” points out Whitehead Institute Member and immunologist Hidde Ploegh.

**FIRST RESPONDERS**

Suppose that the stranger in the elevator was fighting a low-grade infection caused by a virus—a small capsule of DNA (or RNA) that, on its own, has no “life” to speak of. This virus needs a home, and it’s looking to pitch a tent right inside your lung.

This nano-sized capsule of viral DNA has a very specific agenda. Once it lands on a cell, it will work its way into the center of the nucleus, and like a Trojan horse, storm the genome by surprise. From that point on, the cell will do the virus’s bidding. Soon it will replicate the virus, storing inside its membrane a growing mass of viral capsules. The cell will then burst apart, releasing this new batch of viruses into the tissue where they, in turn, will hunt for more cells to breed in.

But your immune system counterattacks. And it does so not as a single monolithic force, but as an entire spectrum of troops united under a single cause.

Many of these troops are ready to
attack from the moment you’re born. Your cough reflex, for one, is part of what scientists call your “innate immunity,” immunity that’s hardwired into your system, no experience required. Your mucus membranes might have also trapped the viral particle in its tracks. Other mechanisms such as fatty acid secretion and even saliva can unleash a biochemical response.

But even those viruses that get lucky and make their way into your lungs run into what’s called the “complement system.”

The complement system is similar to your blood clotting system, where one protein kicks off a cascade of proteins that surround and heal the wounded tissue. In the immune system response, liver-synthesized components (called serum opsonins) recognize the foreign presence and glom onto it, sending out messages to a host of different proteins that also then bind to this foreign invader and begin drilling holes in its outer shell. That viral particle in your lung is now completely disabled. Its coating has been shattered and its internal organs of nucleic acids spill out.

Or perhaps antigen-presenting cells (APCs), macrophages and dendritic cells, stumble over this virus. That scenario is likely, since these highly xenophobic cells constantly patrol the body seeking “foreigners.”

Upon encountering it, APCs will eat the virus, metabolize it, and discharge it as waste.

BUILDING A SMART BOMB

Or you may not be so lucky. The stranger in the elevator may have sneezed out a new strain of flu so virulent that your complement system and macrophages and dendritic cells are always one step behind it. In fact, this strain of flu has gained an evolutionary advantage over other strains for this very reason.

In a few days you’ll be fully symptomatic and entirely miserable as your immune system works overtime trying to play catch-up. These cells know that unless they call in new troops with fancier weapons, they’ll never win.

This newest tactical response is described by scientists as “acquired immunity.” Acquired immunity is found only in vertebrates. It is an aspect of the immune system that develops only in response to a particular invader, but which then provides lifelong immunity to that invader.

“It’s constructed in a more com-
complicated manner and it takes longer to kick in, but once it does, it hits with pinpoint precision,” says Ploegh. In other words, while you spend the next few days at home on the couch wrapped in a comforter watching TV and breathing vaporized water, your body is building a smart bomb.

This smart bomb is composed of two major cell types: T cells, white blood cells that originate in the thymus; and B cells, of which your bone marrow churns out approximately a billion a day.

The messengers that alert these two classes are the APCs we met earlier, which have been feeding on the invaders so heartily.

After a dendritic cell swallows an invader, it digests it into short peptides that are then loaded onto a class of proteins that shuttle them to the cell surface.

Here, the dendritic cells show off chunks of the mutilated virus to their neighbors—in particular, to T cells.

In a landmark paper in Nature, Ploegh’s lab employed real-time microscopy techniques to visualize this process (see “Attack and counterattack” below). The experiment yielded novel images of this occurring in living tissue. It also revealed that when a T cell recognizes the antigen on the surface of a dendritic cell, it can bolster the dendritic cell’s ability to send even more antigen to the surface and thus increase the power of the signal to more T cells. As a result, the alarm is sounded loud and clear throughout your body.

An acquired immunity response takes days to kick in, “but when it does, it hits with pinpoint precision.”

Now the T cells deploy a two-fold tactic. First, they hyper-charge the innate immune system, so that production of its complement and microbial killing mechanisms goes into overdrive. Second, they signal B cells, which charge into the fray and begin producing antibodies, proteins that bind to and thus neutralize this particular virus. These B cells will begin replicating identical copies of themselves, creating a clone-filled antibody serum specially designed to crush this particular virus, and it alone.

It might take a week or so, but these antibodies have built up and amassed in your circulation, and their assault on the virus reaches its height. As they bind to the virus, this further alerts the macrophages, dendritic cells, and even the complement system to mount a larger-scale attack. Soon, the antigen and its progeny are wiped out in your body’s molecular reenactment of General Custer’s last battle.

Once the fighting ends, your body retains the history of this battle. Memory is stored in the form of a rich supply of cloned antibodies that bear the signature of that virus, so plentiful that if it were ever to enter you again, it wouldn’t stand a chance.

**SHAPE SHIFTERS**

Unfortunately, the tale of immunity doesn’t end here.

“Viruses and bacteria have been around a lot longer than vertebrates,” says Whitehead Member and pathogenesis expert Gerald Fink. “If there’s one thing that they’ve learned how to do, it’s to survive.”

Some flu strains, like certain varieties of the much feared avian flu, have evolved to such a virulent degree that our fully deployed T and B cells only get the upper hand in a small percentage of cases. Even more
frightening, other viruses such as malaria and HIV manage to mutate when they’re inside the host, so by the time the acquired immune functions have polished off a strain, a new one has evolved.

Yet another breed of microbes also plays havoc with our immune system: fungus. This threatens transplant or chemotherapy patients who are taking drugs to temporarily subdue their immune systems. If they’re unlucky, they contract a pathogenic hospital-borne fungal microbe.

This microbe knows a few tricks. It can actually alter its outer coating—its skin—so that the few active immune cells will pass right by. The fungus then latches on to tissue, morphs into long finger-like filaments, and causes organ damage. Drugs that target these fungal microbes are particularly brutal on the patient, since fungal cells—unlike bacteria or viruses—are very similar to mammalian cells. As a result, the drugs will damage many healthy cells as well, causing collateral damage that can at times prove fatal.

Kevin Verstrepen, a postdoctoral researcher in Fink’s lab, has discovered a genetic mechanism that enables fungal microbes to disguise themselves so readily (see “Virulence is only skin deep,” page 5).

In a recent Nature Genetics paper, he described how these microbes can dramatically alter their appearance in one cell division, and then change back. The results can be deadly.

Imagine you are immunocompromised and an infection has taken hold. Your immune system attempts a defense with what little strength it retains. Then one fungal cell divides in half moments before it’s attacked. The new daughter cell looks nothing like her mother, but she’s just as deadly. She passes immune cells unnoticed.

“Unfortunately, so far we’ve been unable to develop vaccines against certain pathogens,” says Ploegh. “Flu, HIV, malaria and pathogenic fungi have an evolutionary range that makes them—for now—impossible to wipe out with a single shot.”

Polio, on the other hand, also has a range of “shapes” that it can assume, but they’re limited enough so that a single injection can take care of them all.

INSIDE THE BLACK BOX

Some of Fink’s early work in yeast genetics has been spun off to Microbia in Cambridge, Massachusetts, created by several of his former postdoctoral researchers. Other biotechs also are hammering away at these thorny problems.

But what we know about the epic battles between pathogens and our immune systems is vastly greater than our ability to act on such knowledge. And there is still much more we don’t yet know.

For instance, although we understand much about autoimmunity—in which our immune system turns on us and attacks healthy cells—we actually don’t understand what exactly triggers autoimmune diseases or why this process doesn’t occur more frequently.

And while we know that immune cells recognize invaders by their skin, and we know what happens after they recognize these invaders, the precise mechanisms by which these cells actually “see” each other are still hidden in a biological black box.

“We don’t know the full range of molecules that our immune cells can recognize,” says Robert Wheeler, a postdoctoral researcher in the Fink lab. “And we don’t understand how these cells create the unique signal of ‘Here is a flu virus, attack!’ versus ‘Here is a Candida albicans, attack!’ We know that it happens, but we don’t know how.”

Says Fink, “This is one of biology’s greatest unanswered questions.”
The federal clampdown on funding for human embryonic stem cell (HESC) research and the extraordinary national debate about it haven’t stopped some scientists from pushing ahead in the lab. But they’re barraged by a bewildering and swiftly changing set of laws and regulations. “It’s extremely frustrating to be operating under conditions that do everything to shackle and hobble our research,” says Douglas Melton, co-scientific director of the Harvard Stem Cell Institute.

Of course, the biggest issue comes from President Bush’s executive order, which limits the use of federal funds to research involving 22 approved stem cell lines created before August 2001. That means that the National Institutes of Health can’t fund research on other cell lines, and that researchers should be prepared to demonstrate that their NIH-funded resources don’t stray over that line inappropriately—although NIH has not issued clear guidelines for this. “The issue of how to avoid commingling NIH funding with private funding is a complication,” emphasizes Richard Hynes, a cancer researcher at MIT.

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Meanwhile, a confusing patchwork of state laws ranging from outright prohibitions of embryonic stem cell work to bans on somatic cell nuclear transfer (“therapeutic cloning”) adds further complexity and inhibits collaboration.

This spring did see one major step forward, however, when the National Academies of Sciences released its ethical guidelines for human embryonic stem cell research. The NAS voluntary guidelines, which call for oversight committees called Embryonic Stem Cell Research Oversight (ESCRo), have been adopted by the California Institute for Regenerative Medicine, enabling it to start disbursement of the $300 million a year (for ten years) in state-funded grants. The guidelines don’t address the nuclear transfer issue, but they fill in the loopholes of existing federal guidelines on issues such as requiring informed consent from donors and demanding closer oversight of “chimera” experiments that involve both humans and animals.

The guidelines should make it easier for states to pass their own laws permitting and regulating stem cell research, says Hynes, who co-chaired the NAS committee. “It gives everybody a structure to work from,” he says. “I’m sure it will help states to pass laws.”

Alissa Johnson, senior policy specialist at the National Conference of State Legislatures, says the trend is toward California-style laws that permit nuclear transfer but ban reproductive cloning. Such laws have already been adopted in Connecticut, Massachusetts and New Jersey. “Last year, the majority of legislation that was introduced placed restrictions on HESC research, but this year there’s a growing trend toward permitting activities,” says Johnson. “The hot issue this year is state funding.”

FOLLOWING THE FORMS
Former Whitehead postdoc M. William Lensch is using human ES cells for genetic research into blood-related diseases at Children’s Hospital in Boston. Although working in a newly HESC-friendly state, Lensch faces the ongoing challenge of funding. He is working with three approved stem cell lines, but also has applied to acquire the privately funded lines created by Harvard’s Melton. Those aren’t polluted by mouse cells like the federal lines, and with all 17, he says, “I should have enough to produce a few that really work.”

Harvard has set up strict guidelines detailing how costs and resources should be allocated between federally and privately funded research, and how HESC biological material and its derivatives may be used. In the absence of more complete NIH guidance, many U.S. universities are following these protocols.

Doing so will be “very time-consuming,” says Lensch. “We’ll have to keep this funding really separate in terms of salary and supplies. I’ll have to do everything myself because other people in the lab are not funded to do that. I’ll be doing things like clerical work, keeping track of inventories, and putting stickers on boxes of tissue culture dishes saying that they can’t be intermingled with NIH-supported work.”

The NAS guidelines will help to normalize stem cell research. “We need guidelines and oversight so that people can see that we really are trying to cure disease and not trying to create a cloned army of automatons,” Lensch says. —Eric S. Brown
The battles of Bio 101

As biological knowledge explodes and expectations rise, how are our high school classrooms changing?

“The novels of William Faulkner haven’t changed since he wrote them,” says Maureen Dugan, who teaches biology at Nashoba Regional High School. “Biology has—both what we teach and how we teach it.”

Much of what Massachusetts high school biology students learn today—DNA fingerprinting, the human genome, the Archaea kingdom—wasn’t even known when Dugan and other veteran teachers went to high school. And as teachers struggle to stay current with the juggernaut of biological research, they’re also challenged by national and state educational mandates that often arrive without additional classroom time and resources.

TEACHING MORE

As biological research races ahead, teachers feel compelled to race along. They must teach more material in shorter class periods, often just 45 minutes a day for one semester.

Teachers make tough choices between covering, say, butterflies or somatic cell nuclear transfer. Because cellular and molecular chapters have expanded so much, “nobody gets to the second half of the curriculum about the five kingdoms of life,” laments David Barry, who teaches at Boston Latin Academy with its inner-city population. Teachers cross their fingers that students will remember whatever lessons about snakes, pond water and ecosystems they had in earlier grades.

For many students, a survey class will be the last biology they will ever take. “We get one shot at them,” says Lexington High School’s Susan Offner.

Fortunately, biology’s newsworthiness provides many entry points for learning. “Discussing why embryonic stem cells are controversial goes perfectly with cell division, mitosis and DNA replication,” says Stoneham High School’s Lydia Breen. It also makes science relevant, “and that’s what grabs girls’ attention,” adds Linda McIntosh, who teaches at the all-girls independent school Dana Hall in Wellesley.

But the extraordinary complexity of the science makes it seem inaccessible to less prepared students. “Only the ambitious kids expect to unravel any of this,” says Barry. He wonders how the less ambitious students will be able to demonstrate the proficiency required under the national No Child Left Behind Act.

How do high school teachers themselves keep pace with the rapidly expanding curriculum and changing expectations? “You have to work at it,” says Julie Snyder of Hudson High School. “You can’t be passive.” Many teachers follow research journals such as *Science* and *Nature*. Whitehead’s Teachers Program and other professional development programs also offer “intellectual upgrades.”

TEACHING DIFFERENTLY

National science standards now encourage inquiry-based learning rather than lectures: Students should pose questions, develop hypotheses, and devise and do experiments to test their theories. But many educators say this is hard to do well, and can be an inefficient way to deliver content.

And increasingly, content is what it’s all about on standardized biology tests that loom ever larger. Passing a Massachusetts Comprehensive Assessment System science test will become a requirement for graduation. Teachers worry it will encourage “teaching to the test” and further erode lab time, which they insist is essential to reinforce concepts. Yet the typical 45-minute class is too short for real experimentation.

Stocking labs has gotten harder as school budgets shrink. Even in affluent communities some teachers spend $5,000 of their own money for beakers, frogs or DNA kits.

Can “virtual labs” fill some of the gaps? These interactive computer programs can mimic or, occasionally, actually allow real-world experiments. But most virtual labs aren’t very good or need a lot of tailoring, teachers say. Even good ones serve more as enrichment than as classroom staples. But the Internet itself can provide stimulating opportunities. Offner’s students, for instance, tap a database from the National Institutes of Health to develop phylogenetic trees of organisms isolated from soil samples.

Biology teachers remain passionate about their profession. They ardently hope their students will take away a fundamental understanding of complex issues that will affect their lives. As responsible adults, they say, students will need such biology literacy. How else will they know whether to save their babies’ cord blood, buy genetically modified foods or support embryonic stem cell research?

—Cathryn M. Delude
The genome’s heavy lifters

For the last four years, researchers have had a detailed map of the human genome’s double helix. But having that list is almost like having all the pieces to a large and intricate model airplane—without the instructions. And unless you have some guide to tell you how all the pieces fit together, you’ll never get it off the ground.

Key to the genome “instruction manual” are transcription factors, proteins that adhere to genes and control their activity. Scientists in the lab of Whitehead Member Richard Young have spent the last few years developing technologies that can scan the genome and locate these molecules.

ILLUSTRATION BY CHRISTINA ULLMAN
SCIENTIFIC ADVISOR: CHRIS HARBISON

1. A cell receives a signal, often in the form of a protein that attaches to the cell’s surface. It could be a hormone like insulin, whose ultimate goal is to instruct the cell to store glucose from the bloodstream.

2. After the protein lands on the surface, it sends a message into the cell’s cytoplasm. The signal is typically transmitted in the form of a phosphorylated protein residue. Phosphorylation passes the signal through the cell like a baton, handled by proteins called kinases. The message moves into the nucleus where all genomic information lives.

3. The transcription factor is the final destination of this message. A transcription factor associates with specific genes.
Researchers take a cell and add a chemical that causes each transcription factor (TF) within the cell to become tethered to its respective location on the genome. The genome is then shredded into millions of tiny DNA fragments. The TFs are free-floating in solution stuck to their particular DNA segments.

To locate all the binding regions for a target TF, antibodies designed to adhere to that TF are introduced into the solution. These antibodies are themselves attached to magnetic particles.

Magnets enable the TFs and their bound DNA fragments to be removed from the solution.

TFs, antibodies and magnetic beads are separated from each DNA fragment. Fluorescent tags are attached to each fragment.

The fragments are identified by testing with a DNA microarray, a quarter-sized glass slide covered with thousands of DNA probes.

Eventually, the protein far away on the cell's surface leaves. When the signal has diminished, the transcription factor stops communicating with the polymerase. Expression of the gene is reduced as the polymerase ceases transcription.

Recruited by the transcription factor, the polymerase unravels the gene’s coding region and triggers the mechanism that allows the cell to translate the code first into RNA and then into a protein.

As the transcription factor continues to receive these messages beamed down from the cell’s surface, it then contacts another kind of protein called a polymerase.
Golden days

ON AN EVEN WARMER THAN USUAL

November day, the famed Whitehead Institute formally celebrated its 50th anniversary with a small parade led by local celebrity Alfie, a young clone of philosopher of science Alfred North Whitehead.

The Institute actually was named for benefactor Edwin C. Whitehead, of course. But that escaped the attention of the cloners, who were discovered to operate on a tramp steamer in the Sea of Japan outside the policing of the International Institutes of Health. Alfie was found on the steps of Whitehead seven years ago, along with a bubble chip that included his DNA sequence, a copy of Science and the Modern World, and a fabulous recipe for kimchi.

All five current faculty members who have won the Nobel Prize or Lasker Award were on hand for the festivities.

David Shechner, 54, began his career as a graduate student in David Bartel’s laboratory at the turn of the century. In addition to his groundbreaking work on non-invasive imaging techniques to identify protein assembly and disassembly in individual neurons, Shechner has made inroads into understanding the physiology of thought. “Everyone wanted to see interfaces where you just plugged a wire into the back of someone’s head and they learned French in 30 seconds,” he says, “or how to kung fu fight, like in The Matrix.” (The Matrix was the first in a series of films three decades ago starring California governor Keanu Reeves.)

But Shechner’s research went in a more personal direction. His latest studies have shown it to be possible for one person to directly experience the thoughts and emotional states of another individual, through an ultra-high-bandwidth connection between brain stems. Shechner had hoped that the work would lead to more peaceful interactions among traditionally warring entities who might now better relate to each other. “Maybe we can stop the rock-throwing and the shooting in Gaza,” he says. But the technology thus far has almost exclusively been used in marriage counseling and between Red Sox and Yankee fans, forced to coexist after the teams merged last year and relocated to Hartford.

Ever since the secession of the New England and West Coast states in 2017 to form the Scientific States of America, stem cell research has blossomed.

Fifty-four-year-old Piyush Gupta, who started his Whitehead career as a graduate student of Robert Weinberg, is famous for his work combining neural stem cells with electronic circuitry to create bio-organic sensors. “Certain smells are associated with specific patterns of firing neurons on electronic chips embedded in robotic organs,” he notes. “So we’ve taught robots how to smell. More precisely, we’ve taught ourselves to understand how robots smell.” Drug and chemical weapon detection were obvious applications. But perfume manufacturers and culinary institutes immediately adopted the technology as well, although both such uses were recently made illegal in the French-speaking region of France.

Gupta also teamed up with bioengineers to design scaffolding and reprogrammed embryonic stem cells that, in 2023, successfully grew into individual organs for autologous transplants. Among the refugees who fled the U.S. hoping for medical treatment in Massachusetts was 81-year-old Karl Rove, who believed that he needed a new
heart until doctors discovered that its functions had been taken over decades ago by his gall bladder.

PET THEORIES

Walker Jackson, 59, got his Whitehead start as a postdoc in Susan Lindquist’s lab. Neurodegenerative diseases in humans are mostly a thing of the past, thanks in part to Jackson’s work. His continuing interest in the subject led him in recent years to develop therapies for neurodegenerative diseases that afflict animals, especially pets. “Hey, some people love their pets more than their kids,” Jackson says. “They spend enormous amounts to care for them. And if you’re developing treatments for pets you’re less likely to run into barriers of testing and market applications. Plus, it’s become a de facto testing ground for drugs that can also have human use.”

Investors have indeed boosted research on pet therapies of late, in the hopes of profiting from sales to pet owners and for the modification of the therapies for eventual human use. (See Wall Street Journal, March 15, 2032, “Cat Care Cash Cow.”) Jackson is also working on reverse-engineering the brain, so that replacement parts can be inserted where damage occurred from stroke or other trauma. “If Phineas Gage were alive today, we could insert a computer chip with growth factors that encourages neurons to interface with it and restore his lost function.”

The bar that went through Gage’s head in 1848 is on display nearby at Scientific States General Hospital, near Curt Schilling’s right tendon and the block of ice containing Ben Affleck and Jimmy Fallon, who were accidentally cryogenically frozen while filming the 2009 Farrelly brothers slapstick sci-fi movie Fay’s Transition.

Julia Zeitlinger, who began her Whitehead career as a postdoc in Richard Young’s laboratory, has extended her research into designing microorganisms for specific functions. In the old days, a gene insertion might get a bacterium to produce a desired protein. Zeitlinger’s advances were much more complex, including the one that made her rich and famous—long-lived yeast whose protein products acted on regulatory pathways involved in human obesity and hair growth. “The yeast led to the development of Sam Adams Light and Airy,” she notes with a grin, “the only over-the-counter therapeutic beer for heavy, bald guys.”

Duncan Odom, 62, who also started at Whitehead as a postdoc in the Young laboratory, has been examining the genetic material of the microbes just brought back from the secret mission to Alpha Centauri that was presented to the public as one of the series of lost Mars missions of the 1990s. “The genetic information storage molecule is similar to ours,” Odom notes, “but it seems to code for 30 amino acids instead of 22. Still, the organisms have a distinctively earthlike phenotype, which upon reflection isn’t surprising. Everything in the local galactic area might be presumed to have a common progenitor, spread through meteor collisions that spew hardy organisms into space.”

Odom looks forward to a future finding of completely different kinds of life forms, farther out in the galaxy. “The big discovery will come when we get 50 or 100 light years out, where the earthlike radiation of life starts to peter out. We’re going to find zones where earth-style DNA-based organisms are present along with other critters whose version of DNA has, for example, sulfur-nitrogen backbones, or something else that will immediately distinguish it as being non-earthly. And further away still, you’ll only find the non-earthly stuff. That’s assuming that humanity survives long enough, and society becomes stable enough, to do this kind of exploration, of course. I mean, we have some really smart genetically engineered people out there, but half the world thinks they’re abominations, so who knows which way it all will go?”

Who knows indeed. But as Alfie, quoting his genetic source, noted at the close of the anniversary ceremonies, “It is the business of the future to be dangerous; and it is among the merits of science that it equips the future for its duties.”
You’ve often said there will be another anthrax attack. Why?
It may not be anthrax. There are thousands of other possibilities. The issue with bioterrorism is that it’s easy to have access to these organisms, and it doesn’t take a lot of deaths to cause mayhem. All it takes is fear, and creating fear is very easy. There’s a history of the use of microorganisms in this country, and abroad, to cause this kind of terror, like the cult in Oregon that put Salmonella in the salad bar, or the cult in Japan that used sarin gas in the subway.

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The other piece is that we haven’t figured out who did the U.S. anthrax attack a few years back.

What should we do?
We can do a lot of things to alert the public about possible issues, and the government can try to deal with some of the public health issues. That’s what a lot of this comes down to: public health. And by that I mean alerting hospitals and physicians as to what the symptoms are to these conditions that they rarely, if ever, see.

I’m less worried about anthrax than I am about influenza. I think that we need to worry about emerging communicable diseases. The only cure for that is basic research. We don’t have sufficient sponsorship for vaccine research, so that new strains of influenza, and even new diseases like SARS, are on the rise, and there will be many more. Influenza is one that’s on everyone’s radar now. We need more fundamental research in the biology of these viruses, bacteria, and fungi. And just as importantly, we need to learn how to make better vaccines.

What will that take?
Unfortunately, it may take an influenza outbreak. Available vaccines have in a sense cured and protected us against many of the former plagues. But new diseases are emerging all the time. Flu still kills an enormous number of people. If we tilt the funding too much toward bioterrorism, it won’t solve the basic problem: There are emerging diseases that have nothing to do with bioterrorism and have the potential to kill many more people.

We need to be addressing the very fundamental questions, like, is there a science that would enable us to make new vaccines easily? Now that’s a very pragmatic and practical question. We’re talking about infrastructure. Do we know enough about the immune system and how it interacts with pathogens to say what does and what doesn’t make a good vaccine?

But this is really the best way to protect ourselves against bioterrorism. So the one part is improving the public health, and the other part is supporting basic research so that we’re always one step ahead of naturally emerging organisms, and of those that might be invented. Again, for that we need new antibiotics and new ways to create vaccines.

I’m afraid that at the moment we’re behind the organisms.
Major leagues for microRNA

Tiny ribonucleic acid molecules that regulate gene expression in plants and animals have become big news in biology. Among recent findings, Whitehead Member David Bartel’s lab has discovered that these microRNAs regulate thousands of human genes—more than a third of the genome’s protein-coding regions. The related technique of RNA interference has become a powerful new tool for research, and it offers high promise for clinical use as well. Catch up on microRNA work with our On Topic coverage at www.whitehead.mit.edu/news/ontopic/microrna.html.

Blast from our past

A Whitehead documentary shot in 1987, just five years after the Institute's founding, gives early glimpses of some familiar faces (such as Interim Director David Page) as well as early-stage research that would later pay off in spades. Whitehead Institute: A Community for Discovery is narrated by former Fellow George Daley, then a graduate student in the lab of Founding Director David Baltimore. The documentary follows Daley as he takes part in investigations that eventually produced one of the stepping stones on the path toward Gleevec, the first “designer drug” for cancer therapy. Check out the documentary at www.whitehead.mit.edu/news/academy.

Talking about terror

Founding Member and former Director Gerald Fink has strong opinions about how the U.S. should respond to the threat of bioterrorism (see “The next anthrax attack” on the opposite page). According to Fink, basic biological research is still one of the most powerful weapons that we have. You can hear more on his views about anti-bioterrorism policies and pitfalls in a videotaped talk at the Whitehead Academy, www.whitehead.mit.edu/news/academy.
We live in a world of microbes, like these grown from a human hand print by postdoc Robert Wheeler. You can think of your immune system as an army of defense against threatening microbes. The army provides your body with defense in depth—but recognizing pathogens is one tricky job. See “Signals of war,” page 18.