Power in the blood

Adult stem cells save thousands of patients suffering from blood diseases. Can we multiply their effect?

PLUS
When RNA rules
Silencing cancer
Care and feeding of the young

Of all the species known to modern biology, the one with the longest gestation period is the principal investigator. This is reflected in one famous and frightening statistic: The average age of researchers getting their first National Institutes of Health grant is now 42. Yes, the average first-time grantee is only eight years away from American Association of Retired People membership.

Unsurprisingly, this alarm bell is ringing all over NIH. To its credit, NIH is spending well over a billion dollars a year to address the problem, most recently with Pathway to Independence Awards that will give some lucky postdoctoral researchers their first grants. But the overall situation won’t improve much anytime soon. It’s likely to worsen, given the general budget crunch at NIH (see “NIH goes flat” on page 25). And some argue that the trend toward “big biology” projects further exacerbates the difficulties for young researchers trying to stand on their own.

These young scientists are the non-commissioned officers of biomedicine, the experts who actually grind through the bench work each day for months or years, the lead authors on almost all papers here. And postdocs stay in that role much longer than they did a few decades ago, when pushing back the frontiers of biology was often a quicker exercise. (If you’re working with mice, for instance, each roll of the genetic dice takes you six weeks.)

Last year, a survey by American Scientist found that 58 percent of all U.S. postdocs are between 30 and 35 years old, 69 percent are married or similarly partnered, and no less than 34 percent have children. Most make less than your average house painter. Only a small fraction will ever get a tenured faculty position.

And academic institutions don’t always know how to handle them. Each lab in each institution may set up employment in its own terms, with a unique view on training and mentorship. This lack of uniformity can produce some big surprises. Postdocs who bring fellowships back to their lab, for instance, may lose their benefits in the process, because they no longer are lab employees.

Take this tough period in a researcher’s life, add today’s hammered federal research budgets, and you’ve got a true challenge. For Whitehead and our peer institutions, there’s none larger.

Eric Bender
Acting director, Communications and Public Affairs

On the cover

Hematopoietic cells, the adult blood stem cells found in bone marrow.

Photo by Dennis Kunkel Microscopy.

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Altered nuclear transfer gets proof of concept

Technique produces mouse embryonic stem cells without a viable embryo—but does that change the debate?

Scientists at Whitehead have successfully demonstrated that a theoretical—and controversial—technique for generating embryonic stem cells is indeed possible, at least in mice.

The theory, called altered nuclear transfer (ANT), proposes that researchers first create genetically altered embryo-like entities that are unable to implant in a uterus, and then extract stem cells from these entities. Because these entities cannot implant, they are by definition not “potential” human lives. Some suggest that this would quell the protests of critics who claim that embryonic stem cell research necessitates the destruction of human life. Scientists and ethicists have debated the merits of this approach, but so far it has not been achieved.

“The purpose of our study was to provide a scientific basis for the ethical debate,” says Whitehead Member Rudolf Jaenisch, lead author on the paper published in October in the journal Nature. “Our work is the first proof-of-principle study to show that altered nuclear transfer not only works but is extremely efficient.”

First proposed by William Hurlbut, Stanford University professor and member of the President’s Council on Bioethics, ANT has been described as an ethical alternative to somatic cell nuclear transfer (SCNT), also known as therapeutic cloning.

For SCNT, a donor nucleus, for example one taken from a skin cell, is implanted into a donor egg cell from which the nucleus has been removed. This egg cell is then tricked into thinking it has been fertilized. That causes it to grow into a blastocyst—a mass of about 100 cells—from which stem cells are removed. These embryonic stem cells can divide and replicate themselves indefinitely, and they can form any type of human tissue. But to cull these stem cells, the blastocyst must be destroyed, which critics insist is tantamount to taking a human life.

The procedure theorized by Hurlbut is similar to SCNT, but with one crucial twist: Before the donor nucleus is transferred into the egg cell, its DNA is altered so that the resulting blastocyst has no chance of ever becoming a viable embryo. As a result, a “potential human being” is not destroyed once stem cells have been extracted.

Jaenisch, a firm supporter of all forms of human embryonic stem cell research, has shown that technical concerns about this approach can be overcome.

Jaenisch and Alexander Meissner, a graduate student in his lab, focused on a gene called Cdx2, which enables an embryo to grow a placenta and to form a robust external structure. In order to create a blastocyst that cannot implant in a uterus, the researchers disabled Cdx2 in mouse cells.

They accomplished this with a technique called RNA interference, or RNAi. Here, short interfering RNA (siRNA) molecules are designed to target an individual gene and disrupt its ability to produce protein. In effect, the gene is shut off. Jaenisch and Meissner designed a particular form of siRNA that shut off this gene in the donor nucleus and then incorporated itself into all the cells composing the blastocyst. As a result, all of the resulting mouse blastocysts could not implant.

However, once the stem cells had been extracted from the blastocysts, Cdx2 was still disabled in each of them. To make these cells useful, Meissner deleted the siRNA molecule by transferring a plasmid into each cell. (A plasmid is a unit of DNA that can replicate in a cell apart from the nucleus. Plasmids are usually found in bacteria, and they are a staple of recombinant DNA techniques.) The stem cells resulting from this procedure proved to be just as robust and versatile as stem cells procured in the traditional fashion.

“The success of this procedure in no way precludes the need to pursue all forms of human embryonic stem cell research,” says Jaenisch, who is also a professor of biology at MIT. “Human embryonic stem cells are extraordinarily versatile as stem cells procured in the traditional fashion.

“The success of this procedure in no way precludes the need to pursue all forms of human embryonic stem cell research,” says Jaenisch, who is also a professor of biology at MIT. “Human embryonic stem cells are extraordinarily complicated. If we are ever to realize their therapeutic potential, we must use all known tools and techniques in order to explore the mechanisms that give these cells such startling characteristics.”

ANT, Jaenisch emphasizes, is a modification, but not an alternative, to nuclear transfer, since the approach requires additional manipulations of the donor cells. He hopes that this modification may help resolve some of the issues surrounding work with embryonic stem cells and allow broader federal funding.
Flatworms yield insights into complete regeneration

Key gene is found to regulate the cells produced by planarians’ rather astonishing stem cells

If you take a planarian flatworm and chop it in half, something extraordinary happens: One section grows a new head, the other a new tail, and soon you have two new flatworms. Chop it into quarters, or eighths, and you’ll notice the same thing. For centuries scientists have puzzled over this biological phenomenon, but only recently have they understood that these creatures are a goldmine for exploring how stem cells regenerate damaged tissue.

Now, scientists at Whitehead and the University of Utah School of Medicine have begun to understand how the planarian flatworm achieves complete regeneration of damaged tissue.

“This paper is a starting point for investigating the cellular basis of regeneration,” says Whitehead’s Peter Reddien, lead author on a paper published in November in Science. “Planarians have solved exactly what people want to accomplish with regenerative medicine.”

In May of 2005, Reddien and his then-colleagues at the University of Utah completed the first high-throughput RNA interference screen of planarian genes, finding 204 genes of interest that had corresponding genes in other species, including humans. One of these genes, called smedwi-2, stood out. When smedwi-2 was disabled, the flatworm was suddenly unable to regenerate at all, and its body curled into a stationary, irregular position.

How, then, does smedwi-2 control the planarian’s ability to regenerate? Now, the team reports that smedwi-2 does not regulate the stem cells themselves. Rather, it controls cells produced by stem cells. “Smedwi-2-like genes are found throughout nature, from plants to humans,” notes Alejandro Sanchez Alvarado, professor of neurobiology and anatomy at the University of Utah School of Medicine.
Mad cows and stem cells

What do mad cow disease and stem cells have in common? Quite a bit. The same protein that causes neurodegenerative conditions such as mad cow is also important for helping stem cells in the blood maintain themselves, and for enabling the brain to create neurons.

For over ten years, researchers have known that a protein called PrP causes mad cow disease and its human equivalent, Creutzfeld-Jakob disease, when it forms incorrectly. PrP is a prion, a class of proteins that has the unusual ability to recruit other proteins to change their shape. When a prion changes shape, or “misfolds,” it creates a cascade where neighboring proteins all assume that particular conformation. This, in turn, leads to the fatal brain lesions that characterize diseases such as Creutzfeld-Jakob.

Because the normal form of PrP is found throughout many mammalian species, scientists have reasoned that such a widely conserved protein also must play a beneficial role.

Researchers from the Whitehead labs of Susan Lindquist and Harvey Lodish found that the PrP protein, in its normal state, helps stem cells in the blood to replenish. The scientists compared two groups of mice, one in which PrP was disabled, and a normal control group. Within each group, they extracted bone marrow from one mouse, transferred it to another, then took it from that mouse and transferred it to another, moving bone marrow along like passing a baton. Stem cells were increasingly unable to renew the bone marrow in mice that lacked PrP. The control mice, however, did just fine.

In another experiment Lindquist teamed up with Jeffrey Macklis from Harvard Medical School. The researchers found that PrP affected the rate at which neural precursor cells (a type of stem cell in the brain) create mature neurons such as this.

Researchers alleviate Rett syndrome in mice

Rett syndrome is a debilitating neurological disorder occurring primarily in girls. Currently there is no way to address the syndrome at a molecular level. Now, Whitehead and Brandeis University researchers have dramatically diminished certain manifestations of Rett syndrome in mice, marking a clear path along which to explore possible therapies for people.

Qiang Chang, a postdoctoral scientist in the Jaenisch lab, worked with a previously discovered neuronal gene called Bdnf. Over-expressing this gene in mice caused a drastic reduction in certain Rett symptoms.

The mice were far less lethargic, and activity in the cortical neurons increased. These mice also had slightly larger brains, a longer lifespan and later onset of disease than the other Rett mice.

“This is the first time we’ve successfully reduced the awful symptoms of Rett syndrome using transgenic techniques,” says Jaenisch. The findings were published in the journal Neuron in February.
Cell-based nano machine breaks record

Protozoan coil may offer a powerful model for truly tiny devices

Researchers have known that a long, fibrous coil grown by a single-cell protozoan is, gram for gram, more powerful than a car engine. Now, scientists at Whitehead Institute—together with colleagues at the Marine Biological Laboratory in Woods Hole, Massachusetts—have found that this coil is far stronger than previously thought.

“These findings are twofold,” says Danielle France, a graduate student in the lab of Whitehead’s Paul Matsudaira. “First, they give us an idea of how a cell can manage to generate such enormous force; and second, they provide clues for how engineers might reconstruct these mechanisms for nano-scale devices.”

The spring in the unicellular Vorticella is a contractile fiber bundle, called the spasmoneme, inside a stalk-like appendage. At rest, the stalk is elongated like a stretched telephone cord. When it contracts, the spasmoneme winds back in a flash, forming a tight coil. To find out how strongly Vorticella recoils, France and colleagues used a unique microscope to apply an extra load to the spring. They discovered that the spring could recoil against as much as 300 nano newtons of force.

They also made an important link between the engine’s fuel, calcium, and a major protein component of the stalk called centrin. When the researchers introduced an antibody for centrin into the cell, the spring could no longer contract, indicating that the cell uses a powerful centrin-based mechanism that is unlike other known cellular engines.

France presented these findings at the annual meeting of the American Society for Cell Biology in December.

Okay, what makes a stem cell a stem cell?

Scientists discover key to cells that can create almost any cell in the human body

While we know a great deal of what embryonic stem cells can do, we don’t yet understand how they do it.

Now, researchers at Whitehead working with human embryonic stem cells have uncovered the process responsible for the single most-tantalizing characteristic of these cells: their ability to become just about any type of cell in the body, a trait known as pluripotency.

“This is precisely what makes these stem cells so interesting from a therapeutic perspective,” says Richard Young, senior author on the paper that was published in September in the journal Cell. “They are wired so they can become almost any part of the body. We’ve uncovered a key part of the wiring diagram for these cells and can now see how this is accomplished.”

Researchers in the Whitehead laboratories of Young and Rudolf Jaenisch and the Harvard lab of Douglas Melton focused on three proteins known to be essential for stem cells. These proteins, Oct4, Sox2 and Nanog, are called “transcription factors,” proteins that regulate gene expression.

These proteins were known to help maintain stem cell identity. “But we did not know how these proteins instructed stem cells to be pluripotent,” says Laurie Boyer, a postdoctoral scientist in both the Jaenisch and Young labs.

Using a microarray technology invented in the Young lab, the team identified the genes regulated by these three transcription factors throughout the entire genome of a human embryonic stem cell. The researchers discovered that while these transcription factors activate genes essential for cell growth, they also repress genes needed for an embryo to develop.

“This gives us a framework to further understand how human development is regulated,” says Boyer.
What do newly discovered molecules called microRNAs and the Internet have in common? Both reshaped entire fields in the past decade, says Whitehead postdoctoral fellow Andrew Grimson.

“That’s a fairly grandiose claim for microRNAs,” acknowledges Grimson, who studies them. “But the discovery of the widespread role of these molecules changed the landscape of biology very quickly.”

“Labs across the world, working on a variety of biological questions, are now integrating microRNAs into their research,” says David Bartel, Whitehead Member and Howard Hughes Medical Institute investigator.

Bartel and his colleagues have helped to fuel the frenzy by identifying hundreds of the small RNA molecules and providing compelling evidence that they regulate the production of thousands of proteins in plants and animals.

Until the early 1990s, no one had a clue about microRNAs, which flew under the radar because of their tiny size. Each one contains only 21 to 24 nucleotides, or letters of the genetic alphabet, so scientists simply missed them. Victor Ambros’s group found the first microRNA—lin-4—in 1993 at Harvard Medical School while studying a mutation in the worm *Caenorhabditis elegans.*

Another Harvard researcher detected a second microRNA in 2000. One year later, the floodgates opened with the discovery of nearly a hundred in worms, insects and humans. At this point researchers began calling these tiny regulatory molecules “microRNAs.”

The discoveries changed conceptions of RNA. Scientists have known for decades that RNA molecules serve as messengers and translators, building proteins from DNA sequences. But microRNAs determine which DNA sequences get translated in a given cell, a responsibility once considered the purview of proteins known as transcription factors. MicroRNAs essentially choreograph biological ballets, helping to determine where and when proteins can appear to perform. Thus RNA can add “regulator” to the roles listed on its résumé.

MicroRNAs bind to messenger RNAs that code for proteins involved in activities ranging from development to cancer, and disrupt the production of these proteins. In humans, microRNAs regulate roughly one-third of protein-coding genes, and that’s a conservative estimate.

**GOING THROUGH THE GENOME**

“This is the first discovery of a broad biological mechanism that’s been made since genomics,” says Nobel laureate Phillip Sharp, who is investigating how microRNAs work at MIT, where he is an Institute Professor.

Scientists determined the scope of microRNA activity in a matter of years by mining recently published DNA sequences. Bartel, an RNA biochemist, and computational biologist Christopher Burge of MIT played a leading role. They collaborated to develop computer programs that scanned genomes to identify microRNAs and their messenger RNA targets. Their work helped to ignite interest in microRNAs as biologists in labs around the world realized the tiny molecules regulate a large portion of the protein-
coding genes in plant and animal cells.

“Computational work has produced a very big picture of what microRNAs are likely to be doing in a very short time,” says Sharp. “It feels like the field is moving at warp speed,” agrees Burge, a Whitehead Career Development Associate Professor of Biology. “Genomic approaches have provided a number of important insights, and there has been nice synergy with molecular and biochemical studies.”

**FINDING THE FIRST MICRONAS**

Rosalind Lee and Rhonda Feinbaum, researchers in the Ambros lab, were conducting painstaking experiments on *C. elegans* when they bumped into the first microRNA.

They knew that early development of worm larvae required proper levels of the novel protein lin-14. They also knew that something was regulating those levels and assumed it was another protein, so they set out to isolate the gene for that protein. The result amazed them.

The gene fell on a stretch of DNA once termed “junk” by some, a stretch outside the protein-coding region of the chromosome. It appeared to code for a small RNA molecule—lin-4—that somehow regulated lin-14 levels.

The researchers wondered if lin-4 was an esoteric molecule or a harbinger of a new class of RNAs. “We had no basis for saying that lin-4 was part of something much broader,” says Ambros, who now works at Dartmouth Medical School.

His lab had no luck searching for additional RNAs in the next few years. He was thrilled when researchers in the lab of Harvard Medical School’s Gary Ruvkun discovered another gene in *C. elegans* that coded for a small RNA called let-7 in 2000. In addition to cloning let-7, Ruvkun’s group examined the genomes of a number of other animals and found the gene for let-7 in most of them. The study
foreshadowed the role of genomics in later research.

In 2001, Rockefeller University associate professor Thomas Tuschl (formerly a postdoctoral fellow in the Bartel lab), Ambros and Bartel independently found dozens of additional small RNA genes in worms, flies and humans and decided to call them microRNAs.

LEVERAGING GENOMICS

Bartel realized he needed to look outside the toolbox of classical biology. In 2001, he approached Burge—who had previously developed algorithms to identify protein-coding genes in the human genome—and Lee Lim, who had just completed his PhD training with Burge. The researchers jumped at the chance to explore a new class of genes. Lim worked jointly with the two labs to write a computer program that could scan DNA sequences and predict microRNA genes.

He started by examining known microRNAs. Each microRNA is generated from a piece of RNA that folds back on itself to form a structure that resembles a hairpin. Lim scanned the genome of C. elegans for DNA sequences that would give rise to hairpins after being transcribed into RNA. He then looked for ways to further refine the search.

The double-stranded RNA of a hairpin is chopped and processed into a single-stranded microRNA by proteins called Drosha and Dicer. But apparently these proteins don’t recognize every hairpin. Lim whittled down the list of potential microRNAs by eliminating DNA templates for hairpins that lacked Dicer-friendly characteristics.

Lim then screened the remaining microRNA candidates by comparing the genomic sequence of C. elegans with that of the related worm C. briggsae. He reasoned that most of the genuine microRNAs, those performing critical biological functions, would be conserved across species.

Eventually, the team showed that the human genome contains more than 200 microRNA genes. “We were excited to find new microRNAs,” says Burge. “But then the big question was—what do they do?”

This question had been largely answered in plants. Matthew Jones-Rhoades, a graduate student in the Bartel lab, had discovered that plant microRNAs have extensive and highly conserved pairing to plant messenger RNAs, so he could easily identify many targets of the plant microRNAs.

“At a time when we had about 50 plant targets, we were still in the dark regarding which genes were targeted in animals,” says Bartel.

Benjamin Lewis, a graduate student in both the Bartel and Burge labs, developed a second computer program to bridge this gap. He took the sequences of known microRNAs, scanned animal genomes for corresponding messenger RNA targets and, like Lim, used conservation across species to screen the results. The goal was to find many
more conserved microRNA-mRNA pairings than would result by chance. But the initial program failed to deliver.

The researchers then tried another twist. Previous work showed that some microRNAs pair only partially with their mRNA targets, so the team hypothesized that one part of each microRNA sequence might be particularly important. They were right. Lewis hit the jackpot when he required perfect pairing near one end of the microRNAs. He found tiny sequences, matching short stretches of microRNAs, conserved much more frequently than chance would dictate in the RNAs of mice, rats and humans.

Lewis named the critical stretch that matches targeted RNAs the “seed” of the microRNA. The discovery of the seed gave scientists working on the biochemical interaction between microRNAs and RNAs a big boost. It also allowed Bartel and Lewis to move forward with predicting targets.

They showed that many animal microRNAs have hundreds of conserved targets involved in a variety of processes, and in January 2005, they conservatively estimated that microRNAs regulate one-third of protein-coding genes in humans. This was a shock, as each plant microRNA appears to have just a few targets linked to development.

By the end of 2005, Kyle Kai-How Farh, another graduate student in Bartel’s lab, together with Andrew Grimson, showed there is also a large potential for species-specific targeting, and that in many cases protein-coding genes are evolving to avoid pairing with microRNAs. Thus microRNAs are affecting the majority of human protein-coding genes, at either a functional level or an evolutionary level.

**SPRINGBOARD FOR NEW STUDIES**

While the human genome is clearly full of potential microRNA targets, scientists in the lab have confirmed only a handful of interactions between mammalian microRNAs and RNAs in living cells. Investigators are just beginning to use classical tools to probe the functions of the interactions identified computationally by Bartel and Burge, who are refining their computer programs and designing experiments to test past predictions.

“We’re improving the prediction programs to make them more inclusive and more accurate, and we’re sequencing millions of small RNAs in plants and animals to get a clearer picture of what’s really in the cell environment,” says Bartel.

Graduate student Graham Ruby, for example, is overhauling Lim’s microRNA prediction program. The original application missed many real microRNAs, and Ruby hopes to catch some of the molecules that fell through the cracks. Lim narrowed the list of microRNA precursors by scoring each hairpin according to its microRNA-like characteristics. Ruby adds a new twist. His program includes more than one round of scoring, like the American Idol show. After each round, he eliminates the lowest-scoring hairpins from the pool of candidates. He examines the rejects and uses their characteristics to fine-tune the scoring criteria for the next round, which should make predictions more accurate.

Other researchers in Bartel’s lab are working to determine the mechanism by which microRNAs lower protein levels, as much of it remains a mystery. The picture is clearer in plants, where microRNAs pair fully with and direct the cleavage of messenger RNAs.

But most of the new studies on microRNAs deal with their specific functions. Cancer researchers are particularly interested in the tiny RNAs, as many of them appear to regulate cell proliferation. Several papers last year confirmed this link. Gregory Hannon of Cold Spring Harbor and Scott Hammond of the University of North Carolina, for example, showed that overabundance of a specific group of microRNAs probably contributes to human B cell lymphomas.

“Studies are beginning to show the relevance of microRNAs to human disease,” says postdoctoral fellow Michael Lam, who is working with mice in Bartel’s lab to probe some of the other microRNAs connected to cancer.

“It’s exciting to watch the parallel currents in microRNA research,” says Ambros. “As a classical geneticist, I find it interesting to know how particular microRNAs work in particular situations. But I’m also intrigued by the work of people such as Dave Bartel, who are taking a more genomic view and discerning general patterns of microRNA function and evolution.”

“This will occupy thousands of people for years,” Sharp says. “It will take decades to work out the specifics of many different microRNA-regulated processes and integrate those into whole-organism biology.”

**MicroRNAs on the fast track**

Number of papers published on microRNAs by year

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<td>2001</td>
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**Six microRNAs that are conserved throughout vertebrates appear in purple in zebrafish embryos. This is work by the group of Ronald Plasterk of the Hubrecht Laboratory in the Netherlands, who is among the researchers probing individual microRNAs in their natural contexts.**

*Courtesy of Science*
Controversies—and careers

In the midst of the public uproar about embryonic stem cells, young scientists quietly move ahead with basic research.

Human reproduction is the third rail of U.S. biomedicine, and embryonic stem cell research lives right on that high-current line. Presidential debates, legislation that would criminalize work underway in Massachusetts labs and the spectacular downfall of the world’s most famous stem cell researcher—what exactly hasn’t hit the field in the past few years?

But young researchers keep quietly plugging ahead with the basic research that will unravel the mysteries of these powerful cells. We brought together three postdoctoral researchers and one graduate student, all trained in the lab of Whitehead Member Rudolf Jaenisch, to discuss stem cell politics, science and careers. Participants were Laurie Boyer, Oktay Kirak, Alex Meissner and Konrad Hochedlinger (clockwise from bottom left). David Cameron, associate editor for Paradigm, moderated the discussion.

**MOTERATOR:** How has the stem cell controversy in South Korea affected you?

**BOYER:** I feel that personally I’m obligated to continue to educate the public and to show people that the majority of scientists aren’t driven by this intense desire to be at the forefront regardless of the cost. Most of us are committed to conducting sound and ethical research.

**HOCHEDLINGER:** What I found most surprising is how you can actually make up such a thing. How can someone make up the breakthrough of the century and then try to commercialize the technique?

**BOYER:** But don’t you think that was partly driven by the fact that they were so intent on becoming the world leaders in stem cell research?

**HOCHEDLINGER:** But maybe we should ask ourselves, how could this have happened at all? One of the problems might be that not all of the people involved in this experiment saw the primary data. And that should remind us, when we collaborate with other people, to always request to see the data.

**KIRA:** But it’s also a kind of trust. How much can you trust, and how much do you have to prove?

**MOTERATOR:** Is there a lot of pressure to be the celebrity, the scientific superstar?

**MEISSNER:** It’s kind of fun. (Laughs.)

**BOYER:** I think that sort of thing is somewhat personally imposed.

**MOTERATOR:** Here in this room we have four scientists from four countries. How do you see the U.S. stacking up against the rest of the world?

**KIRA:** Now’s the time when everybody wants to know if somatic cell nuclear transfer works or not on humans. [Also known as therapeutic cloning, and demonstrated in mice, the process promises to eventually aid patients by using their own cells to grow healthy]
tissue.] Everybody all over the world is trying to figure that out.

HOCHEDLINGER: I agree. I think the U.S., in particular Massachusetts, is in a very good situation to answer that question. We have the technology here and the people who can do nuclear transfer. It’s just a matter of time until we know does it work or not.

MODERATOR: Why are you all staying local and not heading for California?

BOYER: Massachusetts is far behind regarding state financial support. But just because someone else comes in and says, “Look, I have this money,” that by itself won’t necessarily move a field forward. If you support the research that’s already ongoing, and support junior scientists to do this research, that’s where you’ll see your best results.

MODERATOR: What’s it like working in a field that many people want banned?

HOCHEDLINGER: It’s a challenge to inform the people. One way to react to these critics is to simply inform them of the advantages this technology has, in particular nuclear transfer, and try to win them onto the other side. When somebody from my family asks me, “What are you doing and what is cloning all about?” I tell them that it has the possibility of treating diseases, such as Alzheimer’s or Parkinson’s, which otherwise would not be treatable.

BOYER: Without further exploration, there can be no gain of knowledge, and we will never know the full potential of stem cell research. This research is still extremely difficult, and clinically relevant results rely on basic science.

MEISSNER: We also need to keep pointing out that no serious scientist is going to reproducively clone humans.

MODERATOR: Have any of you ever felt like you’re investing in a future that might not be legal in a few years?

HOCHEDLINGER: If that were ever to happen, that’s when we’d see scientists leave the country and head to either England or Asia. And that can’t be in the public’s best interest.

MODERATOR: Does the controversy make this research more interesting?

HOCHEDLINGER: Everything that’s novel often is controversial.

BOYER: Science is controversial.

MEISSNER: I see the potential of stem cell research, and that’s what drives me.

BOYER: People are overlooking the basic science. Embryonic stem cells provide a really important opportunity to study early human development. This is incredibly cool. Because of this I tend to insulate myself from the debates. I read about them, but they don’t affect my enthusiasm for the science.

KIRA: This issue is being used as a means to political ends. In the last election, I doubt either candidate really cared that much for stem cell research. Each just tried to spin it in a way that worked for them.

BOYER: A number of years ago people were all up in arms over recombinant DNA technology, frightened over the possibility that people were cloning genetic fragments into vectors that could just be propagated. What if these got into the air? All because it was new. People didn’t really understand what it was.

HOCHEDLINGER: And now everyone is eating genetically modified tomatoes.

MODERATOR: Does the importance of basic research get lost in the larger dialogue?

BOYER: Absolutely. It’s been lost in part in the contentious debate over when life begins. I realize this is a very sensitive subject, but people have used this subjective question over when life begins to argue whether or not stem cell research is ethical or moral. I mean, if you say life begins at conception, day three or day seven, how will that change the basic research?

HOCHEDLINGER: Different people have different explanations for when life, or personhood, begins.

MODERATOR: Has there ever been a time that you heard something from people who oppose this work that has made you reevaluate your position?

BOYER: It’s very subjective. I can’t see ever answering that question in a way that fully satisfies everyone.

MODERATOR: That’s one of the big ethical conundrums here that hasn’t been settled.

BOYER: Absolutely. Do you pay for egg donations? Who are the people who will gravitate toward donating? That’s a huge social issue.

MODERATOR: If you met an influential person who is really on the fence about this whole issue, what would you say?

MEISSNER: I’d ask them, if their child had diabetes and embryonic-stem-cell-based therapy could cure their child, would they do it?

HOCHEDLINGER: I would add just that even if it’s not possible to use it for therapy, we need to keep basic research ongoing. The basic research is invaluable. W
In 1980, a Boston investment bank offered Harvey Lodish $10 million to start a biotech company. To help ponder his choices, Lodish met with seven MIT colleagues who had received similar offers from other firms. They pooled their expertise to form a strategic management consulting group to advise large pharmaceutical companies on the new business of biotechnology. Four years later, they put together a company based on other people’s research about carbohydrates as therapeutics. Now, Genzyme is the biggest biotechnology company in the state and a leader in the industry.

Ten years ago, Paul Matsudaira and his colleagues came up with an idea for building a smaller, faster and cheaper gene-sequencing machine. By the end of this year, the company he co-founded expects to have a forensic genotyping device ready to roll to any crime scene for on-site DNA fingerprinting. In one field test by police last year, Network Biosystems’s mobile lab had DNA results within hours, compared to the usual days or week, which led to the arrest of a suspect 40 minutes later.

Most basic-science discoveries are not suitable for commercialization, more biotechnology companies fail than succeed, and many academics remain suspicious that business interests corrupt the pursuit of scientific knowledge. But Lodish, Matsudaira and other Whitehead Members have carved out an entrepreneurial zone that can defy the odds.

Typically, academic insights translate into useful products when an outside company licenses a university patent. The inventor often becomes an expert advisor especially important to the early stages of developing a drug or device. More than half of Whitehead Members have taken it one step further by co-founding a company, sometimes centered around other researchers’ inventions. That is an extraordinary ratio for any group of biology professors, even at MIT, says Lita Nelsen, director of MIT’s Technology Licensing Office, which formally handles licensing agreements for Whitehead and informally helps scientists meet potential investors and managers.

Their experiences in the business side of science have been shaped by the nature of their research, the extent of their involvement, the rules of engagement, the advice of fellow academics, the skills of their commercial partners, timing and luck. Those experiences, in turn, provide a feedback loop that encourages and nurtures their colleagues’ spinoff efforts.

“There is a vibe,” says David Sabatini, who co-founded Akceli to commercialize a high-throughput microarray technique for investigating gene function developed in part in his lab. “I was influenced by the atmosphere, which acknowledges that, if done correctly, it is the right thing to do.” Akceli has gone out of business, but Sabatini is considering another startup that employs more accurate screening systems to identify drugs targeting a key molecule implicated in a cancer pathway.

Clear rules about keeping scientists’ corporate work separate from their research and teaching work have helped foster a robust entrepreneurial environment, says John Pratt, Whitehead Associate Director.

Whitehead Member Susan Lindquist appreciates the clean line. “A terrible danger in academia is that projects in your lab could be motivated by your financial interest,” she says. “I feel extremely comforted by the very strict boundaries and the experienced environment.”

Two years ago, after waiting in vain for a big pharmaceutical company to follow up on the therapeutic potential of her research, Lindquist co-founded FoldRx. The startup is based...
in part on a yeast-based screening platform to identify small compounds effective against neurodegenerative and other diseases associated with misfolded proteins. Now in its second round of financing, FoldRx has its lead product in phase I clinical trials for safety, and another compound in the pipeline.

**ENTREPRENEURIAL EXPERIENCE**

Another crucial means of smoothing the way for budding businesses is an extensive network of what Pratt calls “intelligent” venture capital firms, which do more than just ante up the money. “We help by bringing dollars and experience and the people who understand drug discovery and development,” says Chris Mirabelli, managing director of Healthcare Ventures, whose investment portfolio includes FoldRx.

“The venture capital people made it much easier,” says David Bartel, who co-founded Alnylam with his former postdoctoral fellows Phillip Zamore and Tom Tuschl, and his collaborator Phillip Sharp (Nobel Prize winner, MIT professor and member of the Whitehead Board of Directors). Harnessing the power of RNA interference to silence genes, Alnylam has one product to treat respiratory syncytial virus in early-stage clinical trials, and a flu program in its pipeline.

“They set it up in a way that wouldn’t take a lot of time away from the lab to manage the company at the beginning,” Bartel says. “It’s something that’s very much done on the side. For us academic researchers, the most important things are still the new scientific results and the manuscripts coming from our labs. Those are the currencies that matter.”

**STICKING WITH SCIENCE**

The potential fiscal rewards for company founders seem to provoke equal parts positive feedback and discomfort. “When money is involved, scientists feel uncomfortable,” Lindquist says. “We could all have made a lot more money becoming surgeons. We went into this profession because we love the science.”

Robert Weinberg, who co-founded Applied bioTechnology 23 years ago to block the RAS oncogene hyperactivity that underlies so many cancers, worries that money can trump the science. “I feel cynical that some biotechnology companies yielded enormous benefits for founders but did little to benefit mankind,” he says. Applied bioTechnology was acquired by Onco- gene Science, which sold its research products division, spun out a diagnostics company, and changed its name to OSI Pharmaceuticals. OSI markets Tarceva, an early success in targeted molecular therapy for cancer.

“There is a lot of money around, but very few good ideas,” says Lodish, who separates his business activities by becoming involved only with companies whose science does not overlap his laboratory science. “At the end of our lives, we don’t want our obits to say we accumulated millions by starting biotech companies, but rather to say we have helped contribute to advances in human health.”

Scientists who start companies can accrue surprising benefits beyond the money, says Richard Young, who has co-founded several companies. “My priority is advancing basic science,” he emphasizes. “But one of the benefits of both being involved in high-quality basic research and in moving the fruits into the commercial arena is that mentors can provide more informed advice about academic and non-academic career opportunities to students and postdocs, and place them in a better position to compete for jobs.”

During a sabbatical, Young helped create some of those jobs with Neo-genesis, a company he co-founded that uses high-throughput combinatorial chemistry to screen for small molecules with therapeutic potential. The firm became part of Schering-Plough. In another plus, he says, “I’ve learned how different fields of endeavor try to implement excellence.”

Peter Hecht affirms the value of training in a commercially savvy place. In 1998, he and three other postdocs left Gerald Fink’s lab at the Cambridge, Massachusetts, firm.

Hecht notes that the Institute’s spinoff skills began with founder Jack Whitehead, a legendary entrepreneur. Whitehead researchers “do this wonderful basic research with a biomedical application,” he says. “They should also be proud of how they have commercialized and turned it into products.”

**Plowing back profits**

When business is good, Whitehead and its inventors benefit from two policies about sharing the fruits of its commercial development.

Like many academic institutions, Whitehead shares one-third of net licensing revenues with the inventors, who earned an extra $250,000 among them last year as a result.

Unique to Whitehead, the Institute receives 20 percent of any equity received by its professional staff as a result of their outside activities. Whitehead has earned several million dollars in the ten years since that policy was unanimously approved by its faculty. Last year was unusually lucrative for Whitehead, due to the acquisition of two privately held companies in which the institute held equity.

Clear rules about separating entrepreneurship from research and teaching have paid off for Whitehead, says Associate Director John Pratt.
Power in the blood

How can we build up the adult stem cells that build your blood?

By David Cameron
But if our alien visitors are confused, it’s understandable. Information about the abilities, and liabilities, of embryonic and adult stem cells has become entirely muddled.

Take, for example, news that came from the University of Minnesota in 2002. Here, researchers led by biologist Catherine Verfaillie published findings that suggested that a certain class of stem cells in the bone marrow, just like their embryonic counterparts, could create a variety of different tissues. These cells, she reported, could form brain, lung, heart, kidney and intestine tissues.

The implications were huge. Embryonic stem cells exist for only a few days in a very early stage after an egg is fertilized. But adult stem cells are sprinkled throughout our tissues and organs, continuously giving rise to new cells. Up until this point, while scientists touted embryonic stem cells as having endless potential to form nearly any kind of tissue in the body, adult stem cells were accorded a single fate on the biological ladder, never to form any tissue other than that from which they came.

But Verfaillie’s findings indicated that adult stem cells might be just as therapeutically useful as their embryonic grandparents. And with no ethical baggage.

“This was probably the biggest thing in adult stem cell development to come along in years,” says Whitehead Fellow Fernando Camargo, then a graduate student at Baylor College of Medicine. “Still, there were some red flags.”

The Minnesota findings showed that mice and rats who had received transplants from cultured stem cells derived from the bone marrow seemed to have cells that genetically matched the donor’s in other tissues, such as liver and muscle. It logically followed then that when these mice received the bone marrow transplant, HSCs made their way into these tissues, listened to their unique signals, and then ripened into liver or muscle cells.

But not everything added up. For example, whether a mouse received a single HSC or 1,000 HSCs from a donor, the number of donor-matching cells ending up in other tissue never changed. “That was illogical,” says Camargo. “If these blood stem cells really were giving rise to other tissue, we should have seen an exponential increase based on the size of the transplant.” In fact, even when blood stem cells were directly injected into liver or muscle, there was still no increase in new cells matching the donor’s.

Camargo was one of a handful of scientists to discover that these HSCs instead were creating mature blood cells that then fused with cells in the liver. In fact, Camargo was the lead author on the Nature Medicine paper that identified macrophages as the fusing culprits.

Today, plasticity of adult stem cells has been largely discredited. Few scientists seriously pursue it (although some, like Stanford’s Helen Blau, are investigating whether fusion itself might have therapeutic value).

The debate roars on, particularly with opponents of embryonic stem cell research. The notion that HSCs can treat everything from liver disease to heart disease to Parkinson’s continues to pop up wherever the debate gets most heated.
While the truth is far more sobering, new findings from Whitehead researchers may help to render HSCs far more therapeutically potent than they’ve been thus far, giving us the ability to treat disease with more precision while sparing patients many brutal side effects.

**TUNING THERAPIES**

Many groups opposed to embryonic stem cell research have made grand claims about adult stem cells, declaring that they can effectively treat brain cancer, neurodegenerative diseases, heart disease, and spinal cord injury, to name a few.

“There are studies out there showing that you can take blood stem cells and get them to do X, Y and Z,” says Orkin. “However, no one can reproduce these studies. Or some of them just aren’t believable. People cite things to prove their preconceived notions.”

The blood system, however, is one area where adult stem cells can boast an unambiguous track record of success.

“We've made a lot of advances over the years using adult stem cells for treating blood-related disease,” notes Camargo, who sees plenty of opportunities ahead.

Orkin, for example, has demonstrated that the genes programming blood cell development are the same ones mutated in leukemias. His lab is investigating how the basic machinery of blood cells interfaces with blood cancers. Camargo is interested in the molecular mechanisms that enable blood stem cells to maintain their “stemness.”

“If you ask us to draw up a list of every gene that’s essential to a hematopoietic stem cell, right now it would be a very short list,” he says. He’s conducting large-scale screenings of these cells using techniques such as microarrays and RNA interference in order to find the key molecular players. His hope is that with such knowledge, scientists can fine-tune these cells for more targeted therapies.

It’s likely that this will happen with blood stem cells long before it happens with any other kind of stem cell.

“We’ve had deep knowledge of the blood system for over 100 years,” says John Dick, director of the University of Toronto’s Program in Stem Cell Biology. “And we’ve understood the major developmental lineages of HSCs since the mid-’70s. Compare that to the liver. Biologists are still arguing over what exactly a liver stem cell looks like.”

As early as the late 1800s, thanks in large part to the Russian biologist Alexander Maximov, scientists knew much about all the different lineages of blood cells. (There are about 12 blood cell lineages, compared to only three cell lineages in brain tissue.) Interest soared in the mid-20th century, when scientists discovered just how vulnerable the blood system was to atomic radiation. By the 1940s the concept of bone marrow transplantation had worked its way into the biomedical world. Bone marrow trials began in the 1950s. But nearly all the patients died.

Canadian researchers James Till and Earnest McCulloch at the Ontario Cancer Institute in Toronto finally identified and characterized the first blood stem cell in the early 1960s (work that has dubbed them the “fathers of stem cell research”). Discovering the proteins that enable HSCs to differentiate and mature, Till and McCulloch made it possible to quantitatively analyze a single hematopoietic stem cell. That revolutionized the success of bone marrow transplants.

Then, in the mid-1980s, Irving Weissman of Stanford University developed methods for purifying HSCs using monoclonal antibodies (antibodies created in mass quantities from a single immune system cell).

“That doesn’t mean that we can make it do what we want. “We've gotten very good at taking blood cells out of one person and transplanting them directly into another person,” says Dick. “But if you want to do something with those cells in culture before transplanting them back, like expand them, we’re still met largely with failure.”

**STUNTED GROWTH**

If there’s one thing that Whitehead Member Harvey Lodish has learned over the last few decades, it’s that hematopoietic stem cells are finicky.

“Our goal is to take these cells out of their natural environment and get them to do in the lab what we want them to do,” says Lodish. “The tricky part is, these stem cells hate being taken out of their natural environment.”

Quite simply, HSCs are happy in the bone marrow. Normally, when you place HSCs in a dish that tries to mimic that environment, they either die, or immediately mature into red and white blood cells.

Scientists would love to maintain HSCs in their stem cell state, multiply their number by 10 or 100, and then transplant them into the patient. “There just simply aren’t enough of them in the bone marrow,” explains Lodish. “The more stem cells you transplant, the more success-
ful the procedure will be. Even in cord blood, the amount of stem cells just isn’t adequate for treating an adult.”

Because of these limitations, bone marrow transplants take a huge toll on patients. For a typical transplant procedure, a patient is first irradiated, which destroys all his or her own diseased bone marrow. A sample of donor marrow is then transplanted into the patient, where it eventually repopulates him or her with healthy blood stem cells.

Unfortunately, because families in the U.S. are getting smaller, only about one-third of the population has related donors. So physicians painstakingly try to match donors with patients so as to minimize immune system rejection. Sometimes after a transplant patients need to take immunosuppressant drugs for months. Other times, they do so for the rest of their lives.

One way to ease immune system rejection is to remove all the T cells from the donor marrow prior to the transplant. (T cells are a kind of white blood cell and are often the first to be recognized as foreign.) And although this effectively deals with the immune rejection, removing the T cells decreases the therapeutic potency of the transplant. In order to increase the potency, you need to increase the number of stem cells coming from the donor. But at the moment, we can’t.

It’s a catch-22: We can give either an effective transplant with immune system complications, or a less-effective transplant without these complications.

“Almost every roadblock we come to with blood stem cells comes down to our inability to multiply them in the lab,” says Lodish.

Over the years many labs have reported success in this area, only to find that these purported advancements have been false leads. But in 2003, Lodish’s lab stumbled upon what might just be the answer.

**SECRETS OF EXPANSION**

Chengcheng Zhang, a postdoctoral researcher, was studying fetal tissue in mice when he discovered a new population of cells that, in the natural environment, appeared to have a preserving effect on HSCs. When he isolated the stem cells and placed them in a lab dish by themselves, they died. When he mixed in these newly discovered cells, the stem cells thrived. But how did these cells manage to sustain the stem cells so dramatically?

Zhang reasoned that they might be secreting certain proteins that sustained the stem cells. Using a series of microarray platforms, Zhang located a number of such proteins.

In the fall of 2003 and early 2005, Zhang reported in the journal *Blood* that one of these proteins called IGF-2, when added to a solution of HSCs, increased their number eightfold. Later he discovered that two more growth factor proteins, angpt12 and angpt13, when combined with IGF-2 into a cocktail, caused a 30-fold increase. These results were reported in *Nature Medicine*.

Lodish is cautiously optimistic. “If these results, which occurred in mice, are repeated with human cells, this will have huge implications for not only bone marrow transplants but for cord blood transplants, for gene therapy and especially for basic research,” he says. His lab now is collaborating with researchers at Lund University in Sweden to repeat these results with human cells.

As Lodish and colleagues continue exploring ways to multiply these slippery cells, others are still trying to discover if HSCs have therapeutic reach beyond blood.

For now, trying to get adult stem cells to behave more like their embryonic cousins appears doomed to failure. When a bigger payoff arrives from these highly specialized cells, it most likely will come from getting them to do what they already do best.
Scientists look at these data and see reason for celebration and room for improvement.

“The good news is there has clearly been a steady increase in the number of female faculty over the last generation,” says Whitehead Member Hazel Sive. “The bad news is a lot of women are still choosing to leave the track that would take them toward faculty appointments or even senior appointments in research.”

In electrical engineering and physics, the low number of women trained is partly to blame for the homogenous faculty. That’s not true for biology. Whitehead Member Terry Orr-Weaver attributes the burst in the number of female assistant professors to active efforts by search committees to recruit women during the past 15 years. But she believes the culture at many institutions makes life tough for female faculty members, who must sometimes fight for local resources and respect. She says the climate becomes even colder for women in senior positions.

“It’s the issue of, Can a man stand to have a woman as his chair?” says Orr-Weaver. “Unfortunately, there are a lot of men for whom that’s very problematic.”

**UNCONSCIOUS BIASES**
This culture could stem, in part, from unconscious biases about the abilities of men and women. A number of studies have demonstrated that people who believe they are unbiased still evaluate men and women differently. In the biomedical field, a research team revealed sexism in the peer-review scoring of postdoctoral fellowship applications by the Swedish Medical Research Council. The team found that a female applicant had to be 2.5 times more productive than a male applicant to receive the same competence score.

“I don’t think all those Swedish reviewers were thinking, This is a woman’s proposal, it’s not going to be as good,” says Whitehead Member Susan Lindquist. “It’s an unconscious expectation that a woman isn’t going to be taken seriously.”

Lindquist says many scientists unintentionally discriminate against women. One of her male colleagues, for example, organized a meeting on protein folding for a select group of scientists and accidentally created a men’s club, as 33 of 35 speakers were men. Lindquist pointed out that women are represented at a much higher level in the field. Her colleague realized his mistake and vowed to do better next time.

Small acts of discrimination accumulate and weigh on women as they move through their careers. The University of Michigan eases the burden by training faculty members to recognize their implicit attitudes toward women. A theatre group also acts out tenure reviews to expose places where unconscious biases come into play.

**THE FAMILY FACTOR**
Although few colleges and universities formally address unconscious biases, some institutions offer daycare and progressive family policies.
to help faculty members balance work with caretaking responsibilities. MIT, for example, runs several childcare centers on campus, facilities that are open to Whitehead employees. But demand outstrips supply, and waiting lists are long. Women typically spend more time than their male colleagues caring for children and elders, so such service shortages hit them harder.

“Women are often responsible for the bulk of childcare and managing a household,” says Orr-Weaver, who is a single mother of two. “If you run an independent research program, you can’t take a break to focus on your other responsibilities because the entire lab is dependent on you.”

Lindquist and Sive, who are also mothers, say institutions can make it easier for female faculty members to juggle all their roles. MIT, for example, automatically adds one year to the tenure clock of a woman who bears a child, an action that was approved in 2001. Fathers and adoptive parents can apply for the same extension. Faculty members can also take one semester off from teaching responsibilities to spend more time with their young children.

Whitehead postdoctoral fellow Astrid Clarke was working on chromosome segregation in fruit flies when she gave birth to twins in August 2003. The next summer, she realized

System shakeup?

Professor Lotte Bailyn of MIT’s Sloan School of Management says scientists should consider alternative structures for labs.

“I can’t imagine why it wouldn’t be possible for two people to share a lab,” she says. “I realize it would be difficult because we have such individualistic criteria for evaluating scientists, but science is becoming more interdisciplinary and collaborative. Current structures may crumble under the pressure."

“The notion of sharing labs to allow more flexible schedules is not new, although it usually involves partners,” comments Whitehead Member Hazel Sive. “I don’t see the whole framework changing soon, though I suspect significant changes could happen when new institutions set up new structures that serve as test cases.”

Learning to lead

After earning her PhD from Harvard University, Hui Ge was excited to join a small group of scientists who move straight from graduate school to their own laboratories. Although the Whitehead Fellows Program launched in 1984, Ge is only the third woman to participate.

“When I was at Harvard, I knew lots of excellent female graduate students who published great work,” Ge says. “But there may not be enough women in the applicant pool to become independent researchers. I think women are sometimes hesitant to go for male-dominated positions when they do not see enough successful role models.”

Ge hopes the trend will change as more women prove they can succeed in such roles. She says the applicant pool will swell further if those women also manage to raise children while running a productive lab—noting the examples of Whitehead Members Susan Lindquist, Terry Orr-Weaver and Hazel Sive.

Postdoctoral researcher Wei Tong is ready to try. Tong, a mother of two young boys, investigates cancer in the lab of Whitehead Member Harvey Lodish and is applying for faculty positions.

Tong knows the next few years will be tough. She has already made sacrifices in her personal life to stay on top of her research. When her second son was born, for example, she took only 10 days off from work, including two weekends. Learning to work more efficiently gave her more time with her children. She considered taking a break from science, but Orr-Weaver, her lab head, suggested she work part-time. A creative arrangement with the American Cancer Society, which funded Clarke’s research, kept her in the lab and on track to apply for faculty positions.

“If I’d taken two or three years off I don’t think I would have been a candidate for faculty positions,” says Clarke.

Whitehead Member Hazel Sive agrees that funding institutions should allow women more flexibility. She would like the National Institutes of Health to give an extra year of funding to every woman who has a child while she is a graduate student or postdoctoral fellow. “I would not like to see a corresponding extra year of funding be given to men who become fathers because the biological burden is not equivalent,” she adds.
In the summer of 2002, graduate student Piyush Gupta came up with an idea about the mechanisms that might drive the growth of melanomas, an often deadly form of skin cancer. In September 2005, he and his colleagues published a paper in *Nature Genetics* that validated the idea. His three-year journey demonstrated how biological research thrives on informal collaborations inside and outside a laboratory, in this case the lab of Whitehead Member Robert Weinberg.

Gupta and his seven collaborators

**SUMMER 2002**
Gupta conceives of the idea for the melanoma project

**FALL 2002**
Starts experiments to inject melanocytes into mice and transform them into tumors with a “cocktail” of cancer genes and compares their metastatic ability with that of other cell types transformed using the identical set of genes

**JANUARY 2003**
First observes metastatic melanoma nodules in vivo in tumor-bearing mice

**APRIL 2003**
Initiates ongoing experimental collaboration with Charlotte Kuperwasser (then a postdoctoral researcher in the Weinberg lab); starts tumor resection experiments, which also indicate that metastasis is not driven by melanoma cell mutations acquired in mice in vivo

**MAY 2003**
Discovers that Slug (a gene involved in both cancer invasiveness and the early development and migration of melanocytes) is expressed in both immortalized melanocytes and metastasized melanoma

**FALL 2003**
Discusses melanoma work with Sridhar Ramaswamy (then of the Whitehead/MIT Genome Center)

**DECEMBER 2003**
Ramaswamy and Jean-Philippe Brunet (also then of the Genome Center) correlate mouse results about Slug with microarray analyses of human moles—the apparent benign precursors of melanomas

**SPRING 2003**
Starts DNA analysis of metastases using Southern blotting, which demonstrates that metastasis of the experimentally generated melanomas probably is not driven by mutation occurring in cells of the primary tumor

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**2002**

**2003**

**The people behind the paper**

Here’s how a string of informal scientific collaborations helped to boost our knowledge of melanomas

By Eric Bender
Photograph by John Soares
demonstrated that human melanocytes, the skin cells that produce pigment, appear to be far more predisposed than most cells to turn malignant because of their developmental history. (See Paradigm Fall, “Why is melanoma so malignant?” on page 3.) Once morphed into cancer cells, melanocytes reawaken a dormant cellular process that lets them scatter throughout the body, with a gene called Slug playing a leading role.

The researchers demonstrated how much more effectively melanoma metastasizes, compared to other cancers, when injected in mice. They proved that this is not caused by mutations in the melanoma tumor beyond those needed to make the initial primary tumor. And they showed that when the melanoma cells are deprived of the Slug gene, their ability to metastasize is strongly inhibited.

As Gupta kicked off the project, he drew on occasional guidance from Weinberg and other Whitehead researchers. As challenges rose, he also tapped into what he describes as “the social network of science,” getting expertise and suggestions from other researchers near and far.

“I had to learn every technique in this paper,” says Gupta, whose undergraduate degree was in math and biochemistry. “There were many technical and experimental hurdles along the way that I could never have overcome without the generous assistance of others in the lab. When experiments did work, I drew heavily on discussions with Bob, collaborators and others in the lab to help me to conceptualize my results.”

“I run a lab that actively encourages people like Piyush to go out and take initiatives on their own, including forming collaborations with other laboratories” says Weinberg. “One of the key powers of our scientific community is an ability to form transient alliances with other research groups in order to get certain projects done. This explains how Piyush accomplished so much in such a relatively short time.”

JANUARY 2004
Stephen Naber (chief of pathology at Tufts-New England Medical Center) analyzes the tissue structure of samples of primary and metastatic melanomas in mice, and observes their similarity to human samples

DECEMBER 2004
Submits paper to Nature Genetics

SPRING 2004
Gupta conducts experiments via RNA interference to test whether inhibition of Slug suppresses in vivo metastasis, which it does

APRIL 2005
Resubmits revised manuscript

OCTOBER 2005
Paper appears in print in journal; Gupta successfully defends his doctoral thesis

SEPTEMBER 2005
Paper appears as advanced online publication

FEBRUARY TO MARCH, 2005
In response to reviewer suggestions, gets help from Joe Gray and Wen-Lin Kuo (at the University of California, San Francisco) to perform comparative genomic hybridization tests (which detect chromosomal copy number changes) on tumor and metastasis samples; these provide a third proof that additional genes in the primary melanoma cells need not be mutated in order for these tumors to spawn metastases

2004

2005
“You quickly can see that there are, for example, 20 genes involved in the response of a cell to a particular drug.”

—TIJN BRUMMELKAMP

Silencing cancer

Thijn Brummelkamp accelerates genetics research with RNA interference screens

By Eric Bender/Photograph by Sam Ogden
Say “benign” skin cancer, and you get an entirely wrong impression. Familial cylindromatosis is a rare disease that doesn’t kill patients, but does subject them to horrible and painful tumors on the head that must be removed regularly by surgery.

Curiously enough, though, there is hope that this condition can be treated quite successfully with aspirin. There’s a striking little detective story behind this discovery, based on a new and powerful biological tool that Whitehead Fellow Thijn Brummelkamp helped to improve.

The tool is RNA interference (RNAi), and it’s a quick and highly flexible method for letting scientists quickly silence a single gene at a time. It also is highly scalable, which is paying off big time in Brummelkamp’s chosen field of cancer research.

Until recently, our advances in understanding cancer have come with agonizing slowness, with scientists painstakingly isolating genes one at a time and then poking around to find how the genes work together and create a cancer-causing network.

In contrast, Brummelkamp can rapidly set up experiments that screen human cancer cells for thousands of suspect genes. “That way we can quickly find new genes that we suspect may play a role in cancer,” he says. Testing for those genes in samples from cancer patients then offers “a very direct approach to finding cancer treatments.”

**STICKING IN HAIRPINS**

RNAi was first demonstrated in mammals around the year 2000 and is now widely used in labs around the world. The process selectively disables gene expression by attacking messenger RNA, the molecule responsible for delivering the gene’s protein recipe to the cell’s machinery.

Originally, RNA interference was created with “short interfering RNAs,” snippets of RNA about 21 base pairs long. Synthesized chemically, these are expensive and short-lived. In 2002, Brummelkamp and colleagues at the Netherlands Cancer Institute demonstrated a powerful alternative.

The Dutch scientists had noted that the biology of siRNAs was very similar to the biology of microRNAs, the naturally occurring short RNA molecules that also affect gene expression by disabling messenger RNAs. (See “When RNA rules” on page 6). Their concept was to get DNA to make hairpin-shaped RNAs quite similar to microRNAs, using common genetic manipulation techniques to do so.

“Other scientists also read the journals, and about 10 groups started to generate a system like this,” Brummelkamp says. “We were the first; that was luck and a bit of hard work.”

They created the hairpin RNAs by using retrovirus vectors (specially created viruses that splice in a segment of DNA). These vectors are easily and cheaply made, replicated and studied with familiar DNA tools. Variations of this technique shot across the biomedical research world; Brummelkamp’s paper is now cited by more than 1,100 other papers.

**PATHWAYS TO PROGRESS**

The research that led to the potential cure for familial cylindromatosis began with ubiquitin, a small protein that aids in demolishing proteins whose time is up.

Brummelkamp and his then-co-workers at the Netherlands Cancer Institute knew that enzymes that helped to add ubiquitin to a protein are important in cancer. They speculated that enzymes that aid in removal of ubiquitin also play a major role.

The scientists took 50 human enzymes involved in removing ubiquitin, and used their RNAi toolkit to measure what happened when the gene for each enzyme was silenced on several signaling pathways implicated in cancers. Activity on the NFκB pathway (which stands for nuclear factor kappa B) shot up dramatically when a certain enzyme was silenced.

This enzyme was known to be mutated in familial cylindromatosis—although its function was unknown. The researchers turned to the scientific literature and found that simple compounds such as aspirin inhibit NFκB.

**DECODING DRUGS**

After joining Whitehead in late 2004, Brummelkamp is now studying how cancer therapeutics work. That’s not at all as well understood as we might hope. “We may know one target or a few targets, but we don’t typically know the whole biological cascade that a drug uses,” he says.

Postdoctoral researchers Alessio Nencioni and Helen Pickersgill are tackling this problem in tests that silence 7,914 human genes. Studying genes on this scale, “you quickly can see that there are, for example, 20 genes involved in the
response of a cell to a particular drug,” says Brummelkamp. Lab work starts by assembling 23,742 hairpin-RNA retrovirus vectors (three for each of those target genes). Each vector’s gene-specific sequence will later act as a molecular “barcode” for DNA microarray analysis. The vectors are introduced into two dishes filled with human cancer cells, where each hairpin knocks down expression of its target gene. One dish is treated with a drug, the other dish is not.

After leaving the cells in culture for a suitable length of time, the researchers harvest surviving cells from each dish, select and amplify their DNA, label the DNA fragments according to their dish of origin, and plop them on a standard DNA microarray. The relative abundance of each “barcode” quickly indicates genes that make cells more or less sensitive to the drug.

The Brummelkamp lab is now employing this strikingly efficient approach to targeting defective mechanisms in cancer cells. “Cancer cells have found ways of turning off the cellular brakes or jamming on the accelerator to grow uncontrollably,” says Brummelkamp. “But their strengths are also hiding specific weaknesses. We can use RNA interference to find those weaknesses and run them off the road.”

A first such experiment involves drugs that inhibit Mdm2, a protein that adds ubiquitin to the p53 protein and thus helps to degrade it. P53 is the king of tumor suppression genes, which is found mutated in about half of human cancers. In the other half of cancers, p53 is not mutated, but the pathway is not functioning well.

Researchers have speculated that in these cancers, inhibiting Mdm2 would activate p53, and it would go ahead with its designated role of suppressing the errant cell. If so, that might lead to powerful therapeutics for these diseases. In a paper appearing in April in Nature Chemical Biology, Brummelkamp and several Dutch colleagues identified a gene that helps to explain why Nutlin-3, a small-molecule drug that inhibits Mdm2, is surprisingly non-toxic to normal cells.

MOVING TOWARD MEDICINE

“If you want to make a conventional drug that inactivates a gene, you can only work with druggable genes,” Brummelkamp notes. “But with RNAi, you can basically inhibit every gene you want.”

Jan Carette, another postdoc in the Brummelkamp lab, is studying ways to inhibit genes that would correct the defect that causes most cases of cystic fibrosis. The disease might be addressed with an RNAi-based inhaler, the researchers speculate.

And labs around the world are delving into other potential RNAi medical applications, studying everything from HIV to, well, hypoallergenic cats. “That’s a nice application of the technology,” Brummelkamp says, smiling. “I’m allergic to cats.”
NIH goes flat
As real funding for biomedical science drops, scientific teams prepare for shakeups
BY RICHARD SALTUS

Blame a changed economy, ballooning federal deficits, tax cuts, the costs of war in Iraq and Afghanistan, Hurricane Katrina, and a shift of research and development dollars toward defense and space exploration.

But real funding for biomedical research is going down. And biologists are facing falling grant success rates, career uncertainty and a bleak outlook for the near term.

For the first time in 36 years, this year’s budget for the National Institutes of Health (NIH) not only failed to grow, but was cut back slightly. In real terms, NIH, which supports more than 212,000 researchers at over 2,800 research universities and medical centers, has less money than it did in 2003.

This trend was underlined in February, when the president’s proposed NIH budget for fiscal year 2007 (starting in October) was flat at $28.6 billion.

Back in the period between 1998 and 2003, in a rare alignment of scientific opportunity and political will, Congress doubled the NIH budget from $13 billion to nearly $27 billion. But those boom times vanished with a bang. In FY 2004 NIH’s budget hikes fell sharply to just 3.2 percent, and that dropped to 2.0 percent in FY 2005. (That was far below the annual rate of inflation in biomedical research and development, which NIH estimated at 5.5 percent that year.)

This year, the small increase initially granted to NIH disappeared in a 1 percent across-the-board cut in federal discretionary spending.

“Our scientists are extremely concerned,” says Jon Retzlaff, director of legislative relations for the Federation of Societies for Experimental Biology.

He says that the percentage of NIH grant applications that get funded is expected to fall to about 20 percent—down from 32 percent in FY 2001. “We’re starting to see young investigators leaving the field to go into private industry and elsewhere,” Retzlaff says.

Whitehead Member Terry Orr-Weaver says that budget reductions “have an almost immediate impact on people entering the field.” And, she adds, “if they see young assistant professors and even established people struggling for funding, it impacts their decision about whether to go into academic science.”

As president of the Genetics Society of America, Orr-Weaver is one of many research leaders who are lobbying Congress to increase the NIH budget. Even if budgets return to historic rates of increase of 7 to 8 percent, damage is unavoidable, she says.

“For established people, having a lapse in funding is an incredible disaster, because you build this team and you need it to keep the project going,” says Orr-Weaver. “If you let people go, the downtime for you is much greater than the time of the lapse in funding.”

Whitehead Member Robert Weinberg argues that NIH must share the blame for what he and others see as a serious threat to the scientific workforce.

NIH’s current emphasis on supporting big collaborative projects, along with the funding squeeze, has made opportunities “so dismal for many young people that they have given up even attempting to launch careers in basic research,” he says.

“NIH has lost sight of the fact that it is far more important to invest in the careers of young people than to fund large research consortia.”
1. A skin cell is taken from a mouse.

2. Short interfering RNA (siRNA) designed to target a specific gene called Cdx2 is introduced into the extracted nucleus. This will prevent the blastocyst from forming a placenta, and it will also cause certain structural difficulties.

3. The nucleus with the siRNA is extracted from the donor cell.

4. The nucleus with the siRNA is injected into an enucleated egg.
How altered nuclear transfer works

Altered nuclear transfer (ANT) has been suggested by some as a potential answer to the ethical debate swirling around therapeutic cloning, or somatic cell nuclear transfer. The idea is that rather than extracting stem cells from an early stage blastocyst, researchers can instead develop an embryo-like entity, one that is unable to implant in a uterus and is structurally unsound. Because such an entity has no chance of ever becoming a viable embryo, a potential life is not destroyed once stem cells are extracted. Using a mouse model, Whitehead researchers have now demonstrated that ANT is biologically possible. Here’s how it works.

The egg cell develops into a blastocyst-like entity, containing inner cell mass. This blastocyst is genetically incapable of ever developing into a fetus.

The Cdx2-deficient cells are isolated from the blastocyst.

Each stem cell is then repaired with a molecule that deletes the siRNA, allowing Cdx2 to activate. The stem cells are now usable and prove to be just as robust and versatile as stem cells extracted from normal embryos.
Reverse brain drain

Should U.S. institutions fear losing talented biology graduate students and postdocs to universities overseas?

For decades we’ve heard about the “brain drain” effect that shuttles promising students from developing countries to the U.S. That shuttle may not be running so well since 2001, but Whitehead Member Harvey Lodish feels that the U.S. still enjoys an edge.

Should we be afraid of a reverse brain drain effect?
At least for biology, the U.S. is still the most attractive place to do scientific research.

Why?
Primarily because of government funding, and a lot of private philanthropy, which we can’t underestimate. When I look around the world, the one disadvantage I see most in other countries is that there’s no private philanthropy. Everything is controlled by the government. Everything is bureaucratic.

We hear good things about England and Singapore—even China.

Well, those countries in particular have a lot going for them. England is developing very rapidly because they have foundations like the Wellcome Trust and Cancer Research UK—lots of private money together with government agencies. But with continental Europe, I’m not so sure. Many of the universities there are paralyzed by bureaucracy.

And Singapore is expanding hugely. The Biopolis [a biomedical research center affiliated with the University of Singapore] is very large and impressive. It recruits internationally. True, it’s bureaucratic, but they have some very smart people running the bureaucracies. The Biopolis is a success.

China’s different. China may be more attractive now than it was ten years ago, but there is currently no university there that parallels any of the top U.S. schools.

If I were an electrical engineer, I’d see vast opportunity in China. By the same token, India is doing a wonderful job training and retaining software engineers. But the life sciences are a different ball of wax.

Biology studies in the U.S. are so attractive to foreign students that we have this huge backlog of postdocs. This results in increased waiting periods and pressure for faculty jobs. Much more is required to get a faculty position than in earlier years because the competition is more intense.

How do the NIH cutbacks affect this?
It’s easy to look at the new NIH budget and say, Woe is me, everything’s falling apart. It is getting tougher. But I’d like to see much more evidence that we’re really losing the most talented people. The top people are going to want to stay here because the opportunities are vastly greater.

Many of the visa problems that occurred after 9/11 seem, for the most part, to have been resolved. There’s now a rebound in foreign applicants to graduate programs, and a lot of interest in foreign students coming here.

So I think it’s a potential problem, but certainly at the high-level institutions we don’t have to worry. I could never imagine a person turning down an MIT appointment in biology for any of these other countries.

Again, I can’t emphasize enough the importance of foundations.

Just walk out of this building and take a look around. You have Whitehead Institute, the Broad Institute, the McGovern Center, the Picower Institute, the Stata Center—all named after philanthropists who care deeply about science. And that’s just something you don’t find anywhere else.
Stem cell scoop

For a snapshot of the latest developments in stem cell research, check out our stem cell page. Here you will find summaries of Whitehead studies of both embryonic and adult stem cells, as well as links to news stories about major research accomplishments from around the world.

Our site also features stories, primers and video presentations that provide background information and place stem cell research in a broader public context. To explore all these materials, visit www.whitehead.mit.edu/news/ontopic/stemcells.

Embryos and ethics

Using mice, Whitehead Member Rudolf Jaenisch and MIT graduate student Alexander Meissner successfully extracted stem cells from a genetically altered embryo-like entity that can't implant in a uterus. NOVA scienceNOW focuses on this pioneering work during an eight-minute segment, exploring the ethical implications of the experiment through the reactions of a clergyman, an ethicist and a prominent stem cell researcher. View the broadcast at www.whitehead.mit.edu/news/ontopic/stemcells.

Taking a quick tour

Since the launch of the Institute in 1982, Whitehead scientists have engineered human cancer cells, sequenced genomes, discovered that prions have a positive side, refined mouse cloning, uncovered the survival secrets of the Y chromosome and more. Take a virtual tour of some selected achievements by visiting our timeline at www.wi.mit.edu/about/timeline.
A class of tiny molecules called microRNAs plays a major role in plant development, as seen in these *Arabidopsis thaliana* seedlings grown by postdoc Allison Mallory. She achieved dramatic results by over-expressing a single microRNA gene. The mutant plants (left and right) have fused seed leaves that form cup-shaped structures. See “When RNA rules” on page 6.