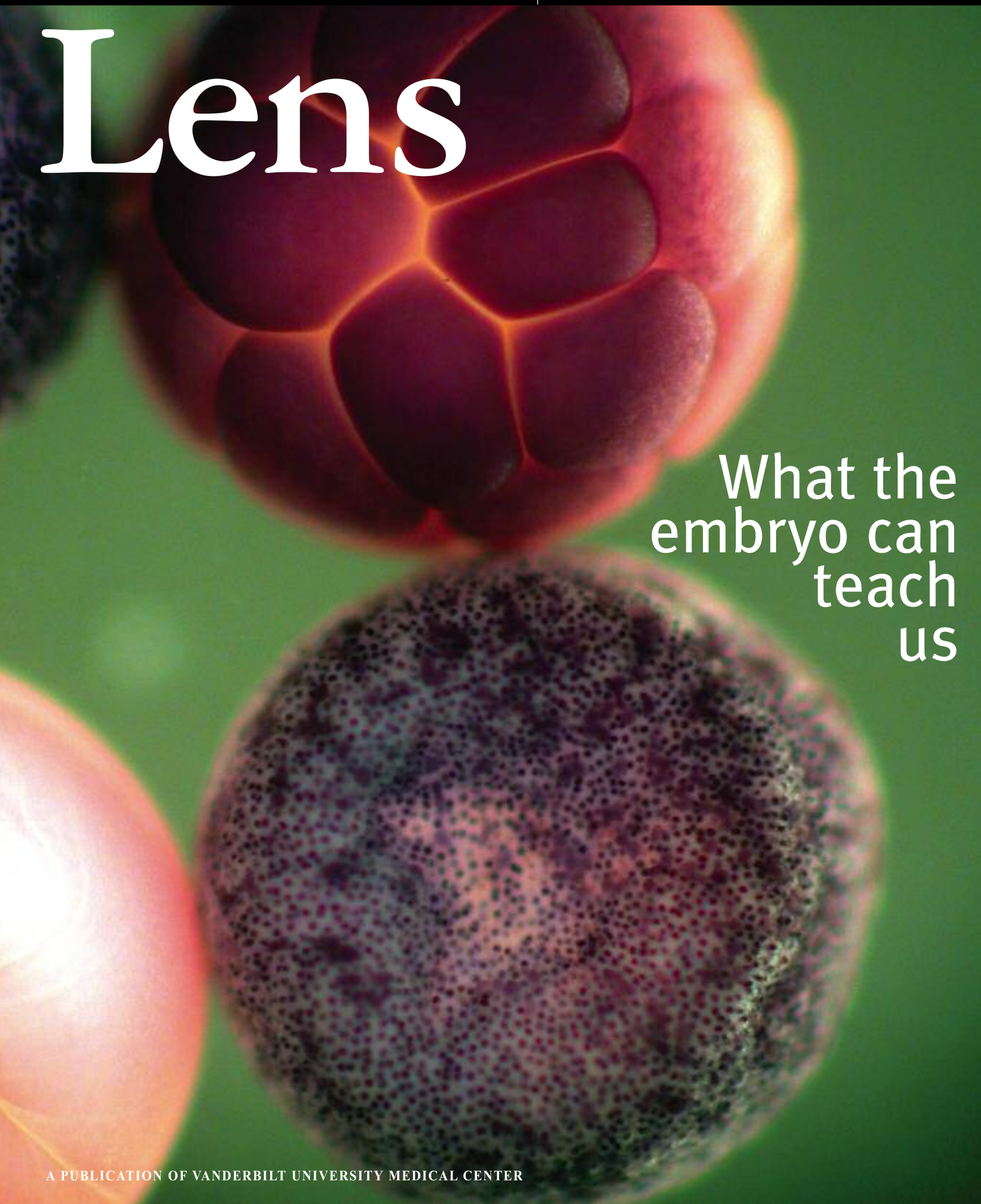
SUMMER 08

p.6 Turning back the clock on diabetes
p.10 The fine art of brain development
p.22 Lub-dub of a healthy heart
p.28 The scientist in society

Lens



What the embryo can teach us

A PUBLICATION OF VANDERBILT UNIVERSITY MEDICAL CENTER

Lens –
A New Way of Looking
at **Science**

SUMMER 2008

VOLUME 6, NUMBER 1

EDITOR
Bill Snyder

CONTRIBUTING WRITERS
Lillian Gu
Lynne Hutchison
Gary Kuhlmann
Leigh MacMillan, Ph.D.
Melissa Marino, Ph.D.
Bill Snyder

PHOTOGRAPHY/ILLUSTRATION
Neil Brake
Dean Dixon
Dominic Doyle
David Johnson
Steve McAlister
Anne Rayner
Jon Roemer

DESIGN
Diana Duren

DIRECTOR OF PUBLICATIONS
Nancy Humphrey

EXECUTIVE DIRECTOR OF NEW MEDIA
Wayne Wood

EDITORIAL OFFICE
Office of News and Public Affairs
D-3237A MCN
Vanderbilt University Medical Center
Nashville, Tennessee 37232-2390
615-322-4747
E-mail address: william.snyder@vanderbilt.edu or
lens@vanderbilt.edu

Cover: ***Xenopus laevis*** **(frog) embryos in early (top) and late (bottom) blastula stages at 20X magnification. Courtesy of Michael Klymkowsky, Ph.D., professor of Molecular, Cellular and Developmental Biology at the University of Colorado at Boulder. The image placed seventh in the 2007 Nikon Small World Photomicrography Competition (www.nikonsmallworld.com).**

Nature is often hidden; sometimes overcome; seldom extinguished.

– FRANCIS BACON

Lens **is published twice a year by Vanderbilt University Medical Center in cooperation with the VUMC Office of News and Public Affairs and the Office of Research. Lens® is a registered mark of Vanderbilt University.**

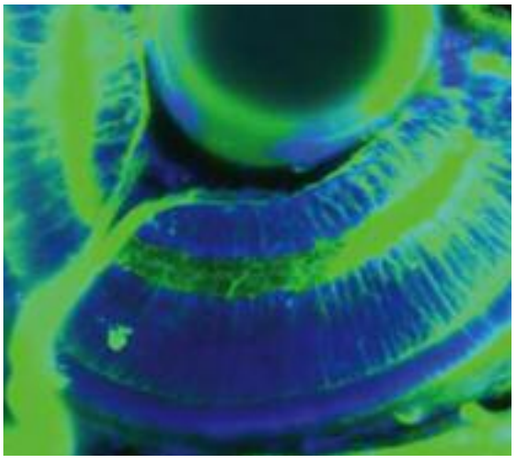
Our goal: to explore the frontiers of biomedical research, and the social and ethical dimensions of the revolution that is occurring in our understanding of health and disease. Through our Lens, we hope to provide for our readers – scientists and those who watch science alike – different perspectives on the course of discovery, and a greater appreciation of the technological, economic, political and social forces that guide it.

Lens

TABLE OF

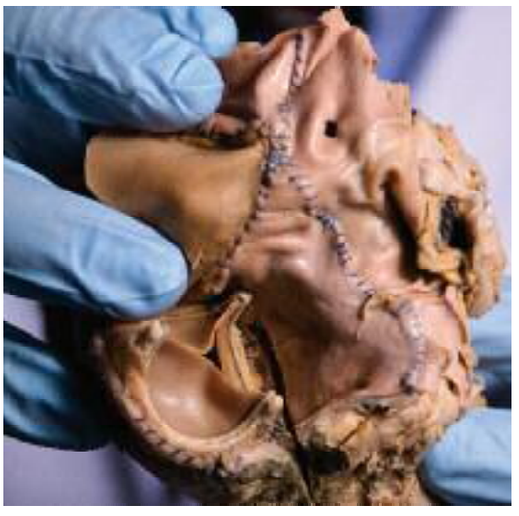
contents

SUMMER 08



page 2 New way of looking at nature

page 16 Two sets of eyes for science



page 22 An alternative to the artificial

2 NATURE'S OPERATING SYSTEM

An essay by Christopher V.E. Wright, D.Phil., director of the Vanderbilt University Program in Developmental Biology

6 TURNING BACK THE CLOCK ON DIABETES

In the not-too-distant future, cells taken from patients with diabetes will be "re-programmed" in the laboratory to create new insulin-producing beta cells that potentially can cure their disease. It sounds like science fiction, but researchers are quickly learning the genetic keys to this developmental path.

10 THE FINE ART OF BRAIN DEVELOPMENT

It takes more than a hammer and chisel to shape the grandest sculpture of them all, the human brain. At least half the genome (10,000 genes or more) may be required. Equally surprising: The living brain never achieves a "final" form.

16 A SHARED PASSION FOR NATURE'S TRUTH

Princeton developmental biologists Eric Wieschaus and Trudi Schüpbach, who are husband and wife, have shared more than their work and a home life raising three daughters – they are of one mind when it comes to preserving the "purity" of scientific truth.

22 THE LUB-DUB OF A HEALTHY HEART

Valves in action give the heart its characteristic lub-dub sound. When they fail, the results can be fatal. What if scientists could build replacement heart valves from patients' own tissues? The hypothesis may be "outrageous," but according to Vanderbilt researchers, it just might work.

28 THE SCIENTIST IN SOCIETY

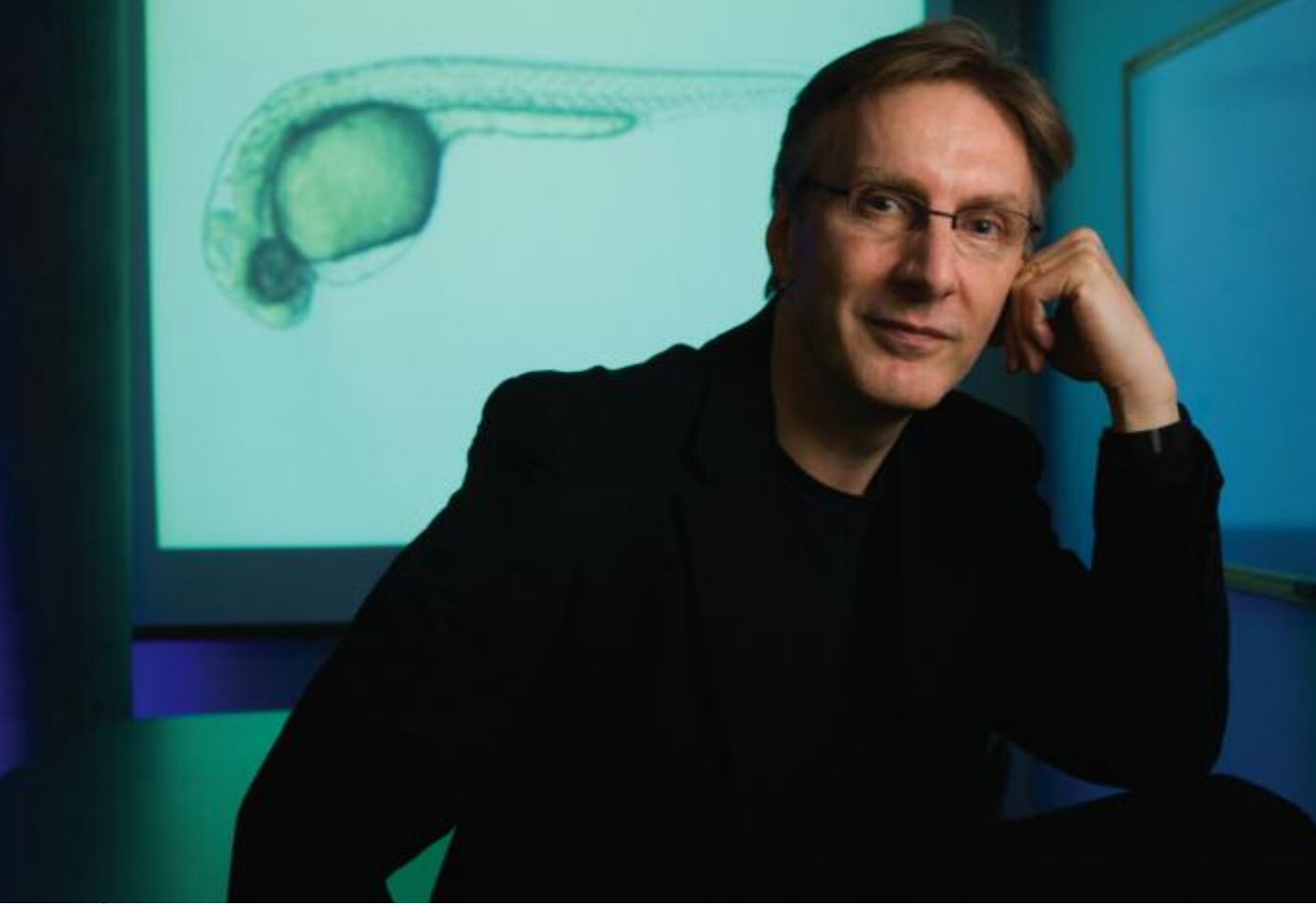
Internationally known reproductive biologist S.K. Dey advocates "a million scientists march" on Washington to sound the alarm about regulatory impediments and dwindling research support that are slowing the pace of progress. If the situation doesn't improve, he warns, scientists may become "an endangered species."

31 RIDING THE NEURAL CREST

The ridges of the neural tube, which close during the fourth week of human embryonic development, may hold the secret to an inherited gastrointestinal disorder that occurs in one of every 5,000 live births in the United States.

32 THE NEXT GENERATION

A unique program at Vanderbilt University connects high school students with scientists. For one graduate, it was a life-changing experience.



Nature's operating system

By Christopher V.E. Wright, D.Phil.

Director, Vanderbilt University Program in Developmental Biology
Professor, Department of Cell & Developmental Biology
Molecular Diabetes Research Professor

What is developmental biology? With respect to human health, why are we so excited about findings being made under this discipline's umbrella, sometimes in model organisms as lowly as tiny worms and fruit flies?

Developmental biology encompasses studies of all organisms, plant and animal, large or small, and whether they are composed of a single cell, clusters or billions of cells. Although focusing largely on early development, embryogenesis and organ formation, in its broadest interpretation the field covers the entire life cycle, including aging and death.

In our modern molecular era, developmental biologists use any analytical technique they can lay their hands on to get a better understanding of the various building blocks and assembly instructions for life: How oocytes and eggs form, how fertilization creates the zygote, and then how embryos in each species develop the right shape with organs all correctly placed.

This remarkably high-fidelity process uses genetic programming to ensure that each organ develops the proper proportions of cell types with complex interconnections to

One day we hope to hold in our hands a comprehensive, minutely detailed catalog of how thousands of molecules, in multitudinous interactions, create neurons or insulin-producing cells.

allow physiological function over what, in humans, can be almost a century of activity.

One day we hope to hold in our hands a comprehensive, minutely detailed catalog of how thousands of molecules, in multitudinous interactions, create neurons or insulin-producing cells. This molecular blueprint is the cell's version of the operating system of the most complicated computer ever built.

This exciting undertaking is of course daunting, even more so because the biological operating system invented by nature is very flexible and versatile. We will need to learn how it changes according to the stage of developmental process, and at a grander level, how it drives evolution. Furthermore, cells are always extremely busy communicating with each other throughout embryonic development and organogenesis, and it is really critical to connect these interactions to the nuclear activity going on in each cell.

By trying to understand how normal development occurs, we also can find out a lot about what can go wrong, with potential for future therapies for congenital disorders, autoimmunity diseases, aging and even cancer. A wide range of animals and organs, and topics, are covered in this issue of *Lens*.

Previous to this current age of completed genome sequences, and before "developmental control genes" had even been found, it was to some people bordering on silly to suggest that organisms all the way from nematode worms and insects, through amphibians, avians and mammals would use basically the same kinds of genes to guide embryogenesis and organogenesis.

This fundamental principle is now, however, relatively well established.

Organisms seem to become more sophisticated by developing new combinations of a basic toolkit of molecules and mechanistic subroutines that control cell formation and interactions. New versions of proteins can arise via the duplication of parts of chromosomes carrying certain developmental control genes, with the protein's sequence and properties then diverging (mutating) slightly. In addition, the gene control sequences that dictate timing and location of protein production can be modified to produce new functions.

Elegant mechanisms

In its beginning, developmental biology was founded upon precise and rigorous descriptive work, a scholarly tradition that lingers to this day. The now-classic experimental embryology of the early 20th century was conducted by a small "club" of the well-to-do who could finance their own inquiries. These pioneering scientists used genetics or direct manipulation to find out how tissues interact to produce the different parts of the embryo.

Today's science, while remaining grounded in precise "descriptology," is more egalitarian, thanks largely to government support. We also have moved into a period where genetics is combined with the most modern biochemical and cell biological methods. Over the last two decades, we have gained the power to assess directly, and in the actual developing tissue, which genes (and even which exact region of their DNA) are being bound to and activated by the development-regulating proteins. I predict that we will soon have amazing multiplex renditions of such information for hundreds of proteins at the same time.

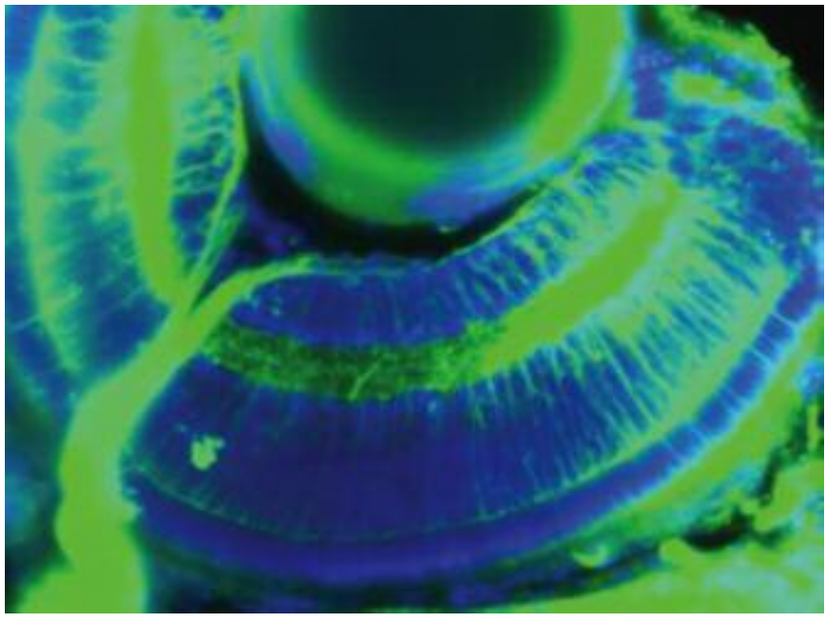
The accomplishments and contributions of developmental biology are easy to recognize in reviewing the list of Nobel laureates in medicine: from the fruit fly geneticist Thomas Morgan (1933) and amphibian embryologist Hans Spemann (1935), to Barbara McClintock, recognized for her work on mobile genetic elements in maize (1983), and Edward Lewis, Christiane Nusslein-Volhard and Eric Wieschaus (see article on E.W. in this *Lens*), for the genetics of fruit fly embryogenesis (1995).

These were followed by Sydney Brenner, Robert Horvitz and John Sulston (2002), honored for their studies on organ development and programmed cell death in nematode worms, and Richard Axel and Linda Buck (2004), for discovering the elegant molecular mechanisms of mammalian olfaction.

Among the most recent Nobel laureates: Andrew Fire and Craig Mello (2006), whose findings in *C. elegans* on gene regulation by short RNAs sparked another revolution in methods for manipulating gene activity; and Mario Capecchi, Martin Evans and Oliver Smithies (2007), recognized for their terrific work on how to engineer targeted mutations in genes using embryonic stem cells.

Discoveries by developmental biologists regarding stem cells stand among the most spellbinding. In their full incarnation, stem cells have almost unlimited proliferation capacity and long lifespan, and their "pluripotency" enables them to put out many different cell types. They have, especially as embryonic stem cells, led to much scientific, political, and ethical/moral debate. Because we are now starting to find out how to control the differentiation of stem cells down specific paths, which could herald the large-scale production of material suitable for cell-based therapies for many kinds of human disease, developmental biologists of all ages (professors and trainees) have a responsibility to get properly informed and to teach others on these issues.

Early stem cell work involved trying hard to generate tissue culture dish models



A section through the eye of a 4-day-old zebrafish larva shows cell nuclei stained blue and neurons labeled green.

Epifluorescence microscopy image by Robert Taylor, graduate student in the Vanderbilt Department of Biological Sciences. Courtesy of Josh Gamse, Ph.D.

for embryonic development, and it was essentially this track that has led to our ability to manipulate the genome in stunning ways. In mice, one can now choose from a delightful smorgasbord of ways to manipulate any chosen gene. These include mutations to inactivate it or to engineer individual amino acid alterations to create models of human syndromes, or to add a fluorescent protein tag that allows us to watch the gene turning on or off in the embryo, and even to pull the cells out very selectively for analysis *in vitro.*

Sense of wonder

There is a justifiable current furor among scientists over the discovery that a special set of just three or four genes can impart the property of pluripotentiality to mature, differentiated cells. This property is usually associated with embryonic cell types. Defining how the necessary changes in gene activity are effected at the level of chromatin organization in the nucleus is an extremely active area of research, with implications at many levels.

Such epigenetic regulation and reprogramming is really attractive, given the prospect of being able to induce regeneration from a patient's own cells to replace those destroyed by Alzheimer's, muscular dystrophy, diabetes, or other diseases. These sorts of discoveries begin to place true regenerative medicine within reasonable grasping distance.

Developmental biology also connects with understanding and controlling cancer. It was a genetic analysis in fruit flies that led eventually to the discovery of a lipid-decorated intercellular signaling protein called Hedgehog, which is also present in mammals, and the dissection of the signaling mechanism by which cells can detect and respond to it.

Hedgehog is multifunctional: it helps define the subtypes and spatial arrangement of neurons in many areas of the central nervous system, and the number and type of digits on the limbs. In some contexts, it controls the degree of cell proliferation, and we have found causative links between defective "always-on" signaling from the Hedgehog receptor and basal cell skin carcinoma.

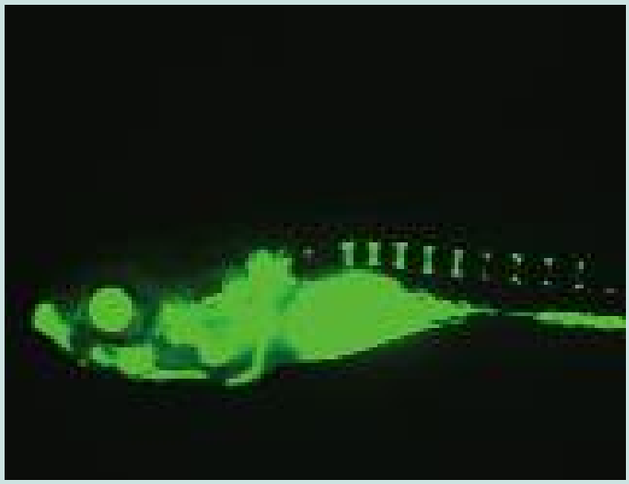
Abnormal activation of other types of intercellular signals also has been found to be central to the development or progression of certain cancers. Among them: signals involving the "Wnt" family of related proteins that are, again, known to play similar roles in fruit flies and vertebrates.

There are many compelling examples to recite among the vast number of discoveries that are relevant to human disease and congenital problems. The articles in this issue cover several of them, hopefully conveying some of the sense of wonder and tremendous discoveries that have been and continue to be made.

I am reminded that it is a deep-seated scholarly drive that often provides a great stimulus toward determining, before anyone else, how a particular process works. Indisputably, some of the most telling discoveries relevant to human health were realized only after years of dedicated and tenacious scientific study. It is hard, however, to predict where the most translatable findings will come from next. In this field, perhaps more than in any other, we have learned over and over again that high quality basic research in esoteric or high-risk areas can often reap unexpected and large rewards.

Meanwhile, those of us in science hope to experience, more than once, that pure rush we feel when, looking down a microscope or learning of others' findings in papers or at seminars, we suddenly see evidence of the totally unexpected way that nature runs things.

In the end, the beauty and economy of biology always stand paramount. **LENS**



The skeleton of a 9-day-old zebrafish embryo glows with calcein, a green fluorescent dye. The bone-staining dye, which the embryo ingested, also was taken up by the eye and intestines.

Image courtesy of Charles Hong, M.D., Ph.D., Vanderbilt University

The power of animal models

BY BILL SNYDER

The march of biomedical science during the past century owes much to a handful of humble creatures, notably the fruit fly, the frog, the worm, the mouse – and recently the zebrafish.

The common fruit fly, *Drosophila melanogaster,* is one of the most-studied organisms on earth, largely because it matures from fertilized egg to adult in a matter of days and is easy to grow and manipulate.

Studies of *Drosophila* and other cold-blooded organisms, including the African clawed frog, *Xenopus laevis*, and the microscopic roundworm, *Caenorhabditis elegans (C. elegans)*, have revealed much about the mechanics of inheritance and development, while the mouse is the most widely used mammalian organism to model many aspects of human disease.

In 1983, in one of the biggest breakthroughs in developmental biology, scientists working independently at the University of Basel and at Indiana University discovered the "homeobox," a stretch of DNA shared by regulatory-switch "Hox" genes in Drosophila that control development of the body segments.

The most surprising discovery about Hox genes is evolutionary. All animals have Hox genes, and nearly all animals use them to determine which appendage should go where along the axis that runs from head to tail. Given that the major animal groups were in place at the start of the Cambrian period, Hox genes must be at least half a billion years old, lending support to Charles Darwin's idea that we all evolved from a common ancestor.

For example, a fruit fly gene called eyeless, which is critical for proper eye formation, is almost identical to a human gene that, when mutated, can result in an *eyeless* baby. Defects in the hedgehog gene signaling pathway, named for the short and prickly "hedgehog-like" fly embryos they generate, also have been linked to several types of cancer in humans.

The difference between us and flies is all in the regulation – more akin to writing new software than to building a whole new computer, or like editing an instruction manual instead of starting over with new instructions.

One of today's up-and-coming animal models is the tiny zebrafish, *Danio rerio*. Its embryo is transparent and develops rapidly: within 24 hours of fertilization, it has a beating heart.

Lilianna Solnica-Krezel, Ph.D., and her colleagues at Vanderbilt University have helped establish the importance of prostaglandin and bone morphogenetic protein (BMP) signaling pathways in zebrafish development.

Prostaglandins are fat-derived compounds that in humans have been linked to pain, inflammation and cancer. BMPs induce formation of bone and cartilage, but disruption of BMP signaling also can affect development of the body plan.

While mice and rats remain important in early drug development and testing, scientists have begun to use zebrafish embryos in screens for new compounds with drug-like activity.

In a recent "chemical genetics" study, Charles Hong, M.D., Ph.D., and his colleagues at Harvard Medical School exposed developing zebrafish to thousands of chemicals to see which might disrupt the dorsoventral (back-to-front) body pattern.

One compound, which they called "dorsomorphin," turned out to be the first selective inhibitor of BMP signaling to be discovered. In mice, inhibiting BMP signaling increases iron levels in the blood, suggesting that dorsomorphin might be useful in treating forms of anemia.

"This work demonstrates the power of chemical genetics," says Hong, currently a Vanderbilt faculty member in Cardiovascular Medicine. **LENS**

Gary Kuhlmann, a freelance science writer based in Elgin, S.C., contributed to this story.

islets of youth

Turning the clock back on diabetes

BY BILL SNYDER

In the not-too-distant future, a child with type 1 diabetes will prick her finger, not to find out if she needs insulin, but to help scientists cure her disease.

Cells from her blood, a scrape of her skin, or another tissue will be "re-programmed" in the laboratory to create insulin-producing beta cells. They'll be injected back into her body in an attempt to repair her damaged pancreas.

"I think we'll be putting pancreatic beta cells that have been made in a dish into people within 10 years," says Mark Magnuson, M.D., director of the Vanderbilt University Center for Stem Cell Biology.

Sounds like science fiction?

Magnuson and others might have agreed – until last year, when several provocative reports were published.

By inserting various combinations of genes, scientists at Kyoto University in Japan and the University of Wisconsin, Madison, reported that they had "induced" human skin cells to revert to an embryonic-like state of "pluripotency" – capable of turning into any other kind of cell.

Injections of these so-called induced pluripotent stem (iPS) cells have been shown to improve symptoms of sickle cell anemia and Parkinson's disease in experimental mice and rats.

Last year also provided evidence that the pancreas can be "coaxed" into repairing itself.

A team of Belgian and French researchers reported that, with the help of a factor called neurogenin3, injured adult mouse pancreas can generate new beta cells from immature "progenitor" cells.

"Everybody had been thinking for the past several years that … you wouldn't make any new 'baby' insulin-producing cells from a progenitor," says Vanderbilt developmental biologist Maureen Gannon, Ph.D. "And now there's evidence that you can reactivate that program. That, to me, is really exciting."

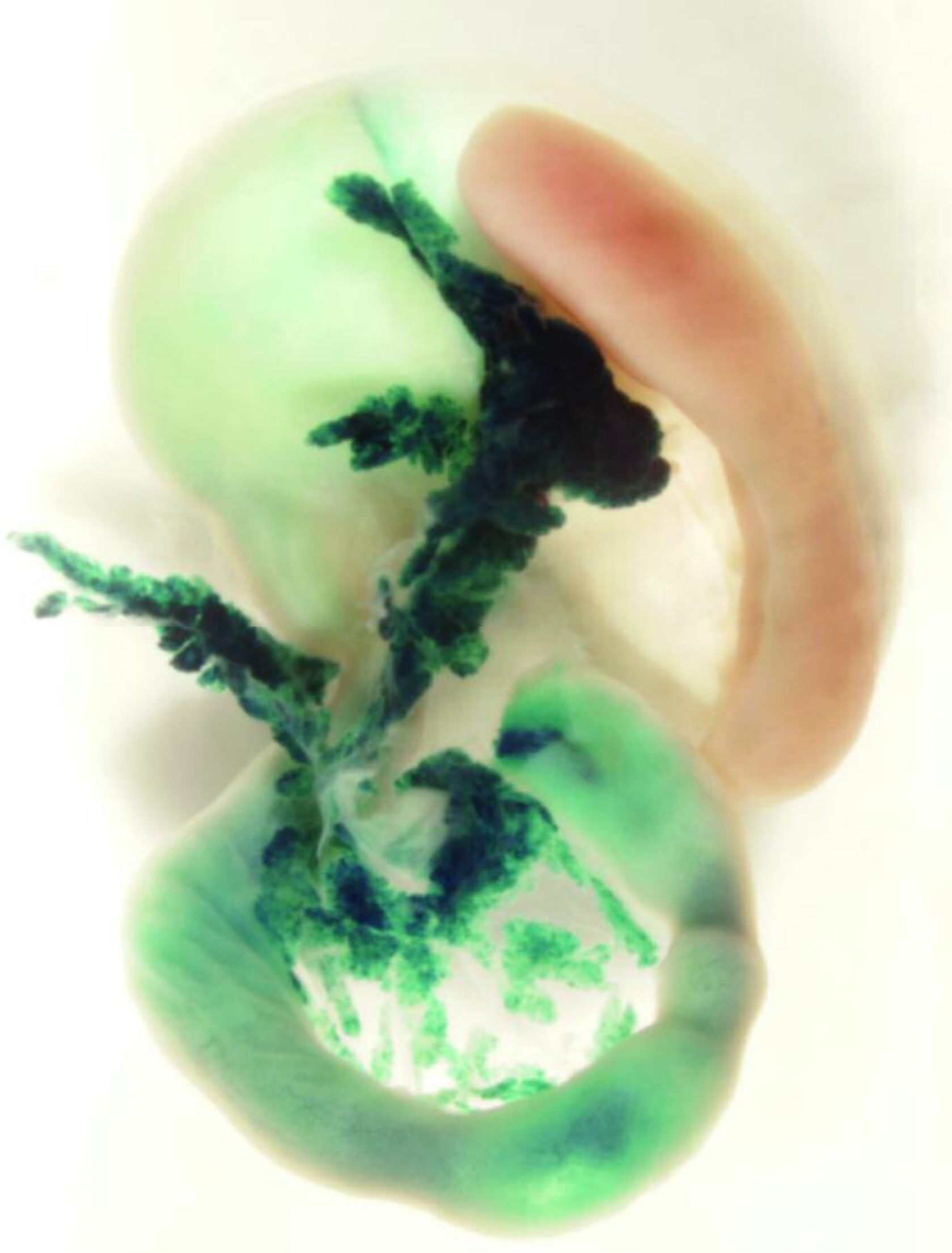
These findings are "hugely radical, unpredicted," Magnuson adds. "They change the paradigm about the plasticity of every cell in the body … (implying that) you can follow the developmental path, go way back to the beginning and then come forward to whatever cell you like."

Underlying problem

Reprogramming a patient's cells to produce insulin would provide a welcome alternative to transplanting pancreatic tissue from other human or animal donors, a procedure limited both by the lack of donor tissue and by the need to suppress the patient's immune system to prevent transplant rejection.

Pictured opposite page: Cells in the pancreas of a 1-week-old mouse that express the Ptf1a transcription factor gene are revealed in this photograph. The *Ptf1a* gene has been genetically engineered to express a bacterial enzyme that produces a dark blue color. In both mice and humans, Ptf1a is essential for formation of the entire pancreas, including insulin-secreting beta cells. By tracing the "cell lineage," or family history, of Ptf1a-expressing cells, scientists hope to learn more about how to maintain – or restore – the function of beta cells. At top right is the sausage-shaped spleen (light orange), and at bottom is the duodenum.

Photo by Fong Cheng Pan, Ph.D., research fellow, Department of Cell & Developmental Biology, Vanderbilt University. Courtesy of Christopher V. E. Wright, D.Phil.



It also could avoid the need to harvest another, more controversial source of stem cells, those derived from human embryos.

However, the virus used by the Japanese scientists to insert the "reprogramming" genes also triggered formation of tumors in mice. "This is not a trivial issue," cautions Alvin Powers, M.D., a leader in the study of pancreatic biology and islet transplantation who directs the Vanderbilt Diabetes Center.

And even if the pancreas can be induced to generate new beta cells, or if skin cells could be "re-programmed" to produce insulin, that does not solve the underlying problem of type 1 diabetes – a misguided attack by the body's immune system that destroys the beta cells.

Christopher V.E. Wright, D.Phil., who directs the Vanderbilt Program in Developmental Biology, agrees.

"What is the nature of the autoimmune problem in diabetes?" he asks. Is the immune system of these patients dysfunctional, such that it mistakes normal tissue for a germ and attacks it? Or could the beta cell be displaying the wrong "badge" on its surface, one that attracts "friendly fire?"

One way to answer these questions is to figure out the steps that lead to the development of the beta cell, and then to try to determine whether that differentiation program is "messed up" in the patient with diabetes.

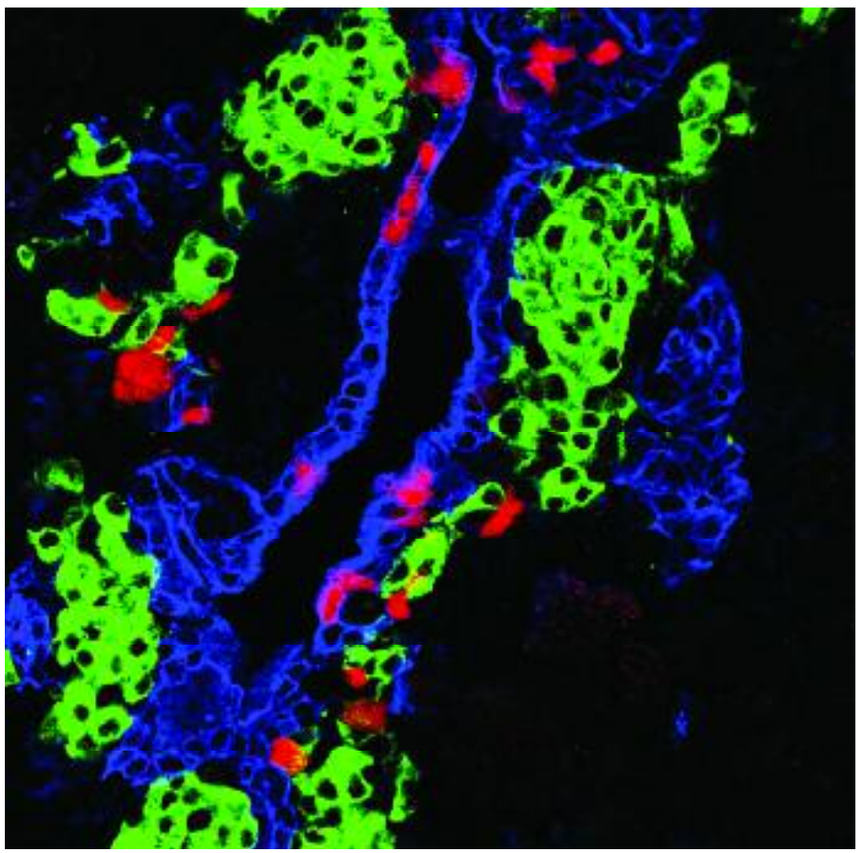
That's where the embryo may help.

During a part of embryonic development called gastrulation, groups of cells migrate into three distinct layers: the outer layer or ectoderm, which will develop into the nervous system and skin; a middle layer or mesoderm, which will become the musculature and other internal organs; and an inner layer, or endoderm, which will form the stomach, intestines, liver – and the pancreas.

The human pancreas secretes digestive enzymes and, from cells clustered in the islets of Langerhans, several important hormones, including insulin.

One of insulin's main jobs is to ensure that fuel – primarily glucose – gets from the bloodstream into the tissues. Diabetes, characterized by a sustained and dangerous rise in blood levels of glucose, occurs when insulin production is unable to keep up with demand.

Whereas in type 1 diabetes there is a loss of beta cells, in type 2 diabetes, the most common form of the disease, the tissues of the body have become "resistant" to insulin. The beta cells also have lost their ability to produce sufficient levels of the hormone.



Colors hint at the "cell lineage" in the developing pancreatic tissue of a mid-gestational mouse embryo. Antibodies linked to fluorescent molecules that absorb and re-emit light of different wavelengths detect hormone-producing endocrine tissue (green), epithelial duct and associated progenitor cells (blue), or cells (red) that specifically express pancreas specific transcription factor-1a.

Image by Fong Cheng Pan, Ph.D., research fellow, Cell & Developmental Biology, Vanderbilt. Courtesy of Christopher V.E. Wright, D.Phil.

Wright believes developmental biology may hold the keys to unlocking the mystery of this ancient disorder.

"One of my strongest beliefs is that developmental biology and cancer biology and aging and all forms of inherited disease are basically the same process," he says.

"Because the study of developmental biology involves trying to understand the generation of life, it uses and develops completely novel principles and tools and ways of looking at things to understand how multiple signaling pathways are used by cells to talk to each other in complicated ways.

"And because of that, it ends up being one of the most pioneering of disciplines."

Pioneering discipline

Until the early 1980s, the mechanisms of development had been shrouded in mystery. Then, as the new tools of molecular biology became widely available, came several pivotal discoveries.

Among them: the discovery of the "homeobox," from the Greek word for similar, a specific short sequence of DNA shared by a set of regulatory-switch genes in the fruit fly genome that determine which embryonic segments will become the future head, thorax and the abdomen. Nearly identical sequences were found in the genes of vertebrates, including mice and humans.

At the time, Wright was a freshly minted biochemist from Oxford University who had just joined the laboratory of pioneering developmental biologist Edward De Robertis, M.D., Ph.D., at UCLA.

"I had somehow a gut feeling that the homeobox genes were a huge breakthrough," he recalls.

De Robertis set Wright to work on the frog *Xenopus laevis*. By 1988, they had discovered the first homeobox gene expressed exclusively in the endoderm.

The gene, eventually named *pdx1*, for pancreatic and duodenal homeobox factor 1, is essential for development of the pancreas – as well as for maintenance of the adult beta cell. The *pdx1* gene

encodes a protein, called a transcription factor, which turns on other genes.

Wright's career was launched at a time when scientists were just learning the "language" of the cell. He came to Vanderbilt in 1990 to work with Brigid Hogan, Ph.D., now chair of Cell Biology at Duke University, who helped pioneer methods for introducing extra genetic material into mice embryos.

Another technique, gene targeting, enabled the Vanderbilt team – which by then included Magnuson, Roland Stein, Ph.D., and Patricia Labosky, Ph.D. – to study what happens to the pancreas when pdx-1 is "knocked out" of embryonic stem cells in the mouse.

Since then, Wright and his colleagues have continued to elaborate the role that *pdx1* plays in pancreas development. Among their findings:

• When one of the two copies of the *pdx1* gene normally inherited from one's parents is inactivated in mice, the animals exhibit a pre-diabetic state in which blood glucose levels are higher than normal. Similarly, in humans, certain mutations in the gene are associated with increased risk for developing a form of type 2 diabetes.

• Both *pdx1* and another regulatory gene for pancreas specific transcription factor-1a (*Ptf1a*) signal progenitor cells to become pancreas. When *Ptf1a* is inactivated, however, these cells instead form the lining of the duodenum.

Two other transcription factors – HNF6 and FoxM1 – studied by Gannon and her colleagues play important roles in development and maintenance of pancreatic function.

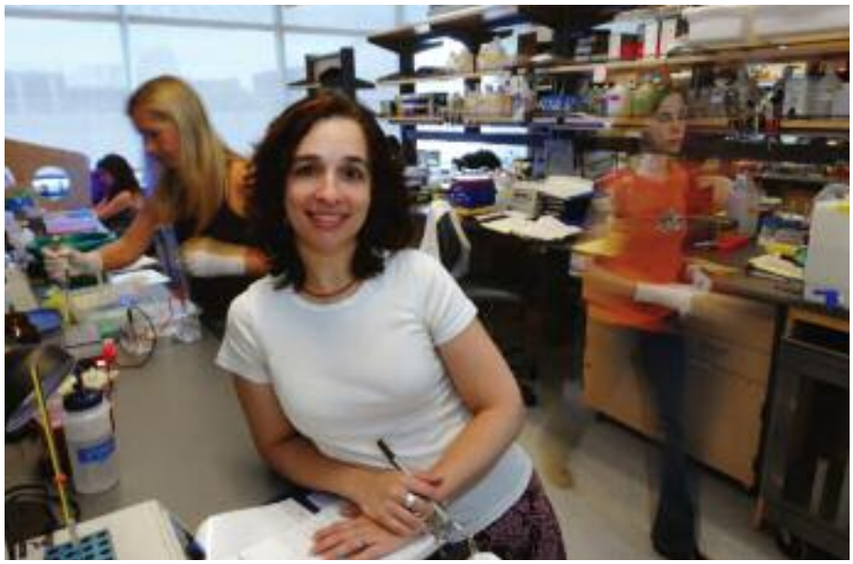
HNF6's role is time sensitive: unless its gene is turned off at a critical stage, the pancreatic islets fail to develop normally in mice. FoxM1, on the other hand, is essential for expanding the population of insulin-producing beta cells after birth. Mice lacking the *FoxM1* gene are born with normal pancreases but slowly lose beta cells and end up with diabetes.

These factors "are all connected, but we haven't filled in all the lines," says Gannon, associate professor of Medicine, Molecular Physiology & Biophysics, and Cell & Developmental Biology.

Brand new view

Meanwhile, Magnuson had become interested in a variation of the knock-out technique that uses a DNA-cutting enzyme called Cre recombinase.

Because the enzyme cuts at precise locations in the DNA, this method enabled Magnuson and his colleagues to inactivate various genes involved in insulin action and glucose regulation in specific tissues, notably the pancreas and liver – to figure out exactly what they do. A major goal now is to learn all the steps needed to direct a stem cell to become a beta cell.



ANNE RAYNER

Maureen Gannon, Ph.D., in her Vanderbilt lab. Behind her, from left: graduate student Kathryn Henley, research assistant Christine Pope, and Magda Bokiej, a student in the Medical Scientist (M.D./Ph.D.) Training Program.

Wright visualizes a day when scientists will be able to create "personalized" pluripotent stem cells from the tissues of a patient with diabetes, and then kick them forward to see if they develop into beta cells completely normally, or display abnormalities at a specific stage of formation.

"That is what stem cell biology has done for us so far," adds Magnuson, the Earl W. Sutherland Jr. Professor of Molecular Physiology & Biophysics. "It has given us a brand new view of what is possible."

It also has spurred collaboration across diverse research disciplines.

For example, Vanderbilt scientists as diverse as David Piston, Ph.D., who helped pioneer the use of fluorescence imaging to study living beta cells, Richard O'Brien, Ph.D., who studies diabetes-related genes, and Guoqiang Gu, Ph.D., an expert on Cre recombinase, compare notes with Powers, Magnuson, Wright, Gannon, Stein and their colleagues in a weekly Beta Cell Biology Interest Group.

On a regional level, Stein recently organized the first annual meeting of the Upper Midwest Islet Club at Vanderbilt to foster communication between senior and junior investigators, with "a decided focus" on graduate students, post-doctoral fellows and new faculty members. The goal: to inspire the next generation of researchers.

Vanderbilt also is the coordinating center for the international Beta Cell Biology Consortium, established in 2001 in response to a congressional mandate to capitalize on the advances of the previous two decades.

Currently the consortium facilitates collaboration among 30 principal investigators from nine countries, three of whom reported earlier this year that, in the mouse at least, "you can reactivate the embryonic program and make new insulin-producing cells in adult pancreases from a progenitor cell," Gannon says.

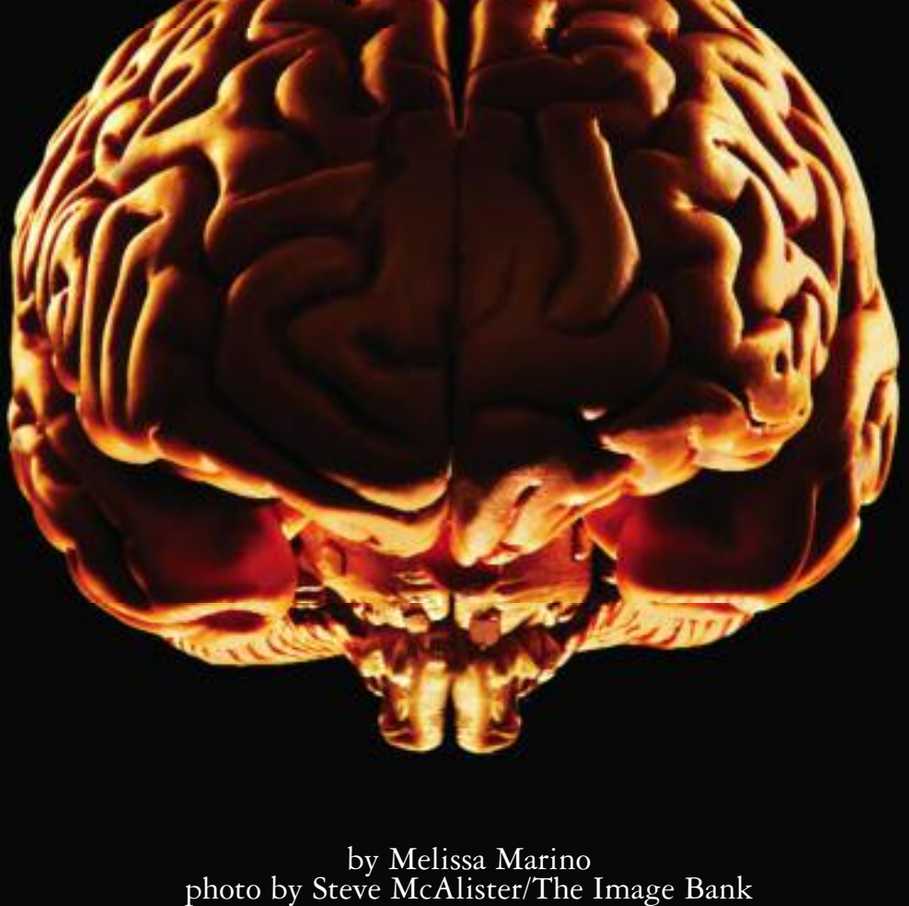
While collaboration is no guarantee of faster progress, "I would not be at all surprised if five years yields a completely novel way of looking at cells," says Wright, whose vision of the future draws from the science fiction comic books of his youth.

"I think there will be a way of either looking at a normal cell or labeling a cell in some very clever way, and then probing the cell, with something like a ray gun," he continues, his eyes twinkling.

That futuristic firing will provide "extremely high-precision data telling us what is going on inside all the cells – all the protein-protein interactions, metabolic pathways and which genes are being switched on and off, and in real time."

"You ought to be able to pull a trigger," Wright envisions, "and get really exciting information directly from each nucleus." **LENS**

THE FINE ART *of* BRAIN DEVELOPMENT



by Melissa Marino
photo by Steve McAlister/The Image Bank

From rocks that took nature eons to build, some of the world's most notable sculptures – Michelangelo's "David," Rodin's "The Thinker," Mount Rushmore – have emerged, their basic shapes roughed out with chisel and mallet, and their fine detail and subtle textures carefully carved and refined with more delicate tools and a lighter hand.

Perhaps the grandest sculpture of all, the human brain, is shaped by a combination of these basic processes – an early "building up" of the brain's bulk, followed by a "roughing out" of the major brain regions, and later, a meticulous refinement of the detail that imparts its unique functions.

Sculpting a brain – or indeed, an entire nervous system – takes a lot more than a hammer and chisel, requiring at least one-half of the entire human genome. The end product, a grayish-pink, 3-pound gelatinous mass, may be the most complex structure in the known universe – containing at least 100 billion nerve cells (neurons) and 1 trillion support cells (glia), which can make at least 1 quadrillion connections between them. The perhaps hundreds of different chemicals (neurotransmitters) that relay information between these neurons further increase the complexity.

It's no wonder that many questions about how the brain develops – both normally and abnormally – remain unanswered. How is the incredible diversity of brain cells and connections generated from our finite genome? How do the maturing neurons know where to go and which neighbors to "hook up" with? And how do events during development affect the brain's ability later in life to acquire and store new information through rewiring (plasticity)?

Using a range of animal models from fly to mouse, Vanderbilt researchers across a number of disciplines are probing the many mysteries of brain development and are providing insights into how it may go awry in neurological disease.

"Normal brain development is a staggeringly beautiful and wondrous thing," says Kendal Broadie, Ph.D., Stevenson Professor of Neurobiology and professor of Biological Sciences and Pharmacology at Vanderbilt University. "It gives rise to this structure that's beyond our comprehension – a structure that allows you to see, think, run and sing."

This remarkable structure begins to emerge from a single layer of neural stem cells lining a tube in the early embryo at around the third to fourth week of gestation in humans.

Thus begins the "build up" phase of brain sculpting. The cells lining the wall of this neural tube begin dividing rapidly – by some estimates, at the rate of 50,000 cells per second – and the walls progressively thicken. Soon, decisions are made as to whether these primitive cells go on to become neurons, the cells that process and transmit information, or glial cells, the supportive "partner" cells that provide nutrients, oxygen and other necessities to neurons.

As primitive nerve cells become neurons, they develop extensions from their cell bodies – many short projections called dendrites that receive incoming signals, and a single, long axon that transmits those signals to the next neuron.

Glial cells, though they have many similar features to neurons, do not develop these specialized appendages. Instead, some of them go on to form the protective sheath called myelin that wraps and insulates the axons of many neurons and enhances the speed with which nerve impulses can travel from cell to cell.

GLOWING GENES

Bruce Appel, Ph.D., associate professor of Biological Sciences at Vanderbilt, is studying the development and specification of oligodendrocytes, the glial cells that form myelin in the central nervous system (CNS), which includes the brain and spinal cord.

In humans, myelination begins shortly before birth and continues into adolescence. In Appel's research subject, the zebrafish, myelination starts around the third day after fertilization.

The zebrafish is a great model system for studying nervous system development, Appel says, because the embryo is transparent and develops entirely outside the mother. And it develops in two days. By comparison, the mouse embryo takes about 10 times longer to mature.

By engineering certain zebrafish genes to glow green, Appel can easily view specific sets of neural progenitor cells – immature nerve cells – and in particular, the cells that go on to produce oligodendrocytes.

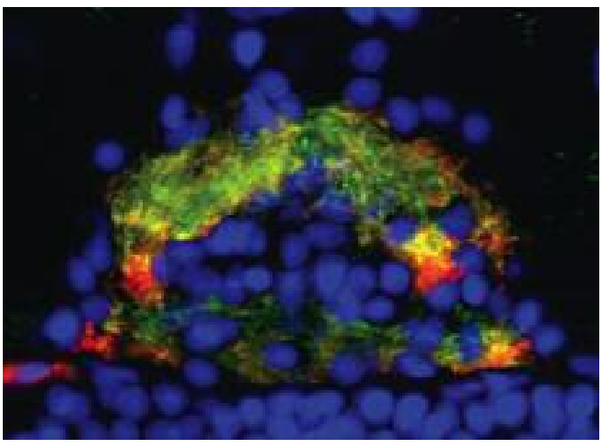
"We've found that oligodendrocytes, which were always considered to be really boring cells, actually turn out to be incredibly dynamic," he says. The cells send out fine processes, called filopodia, and appear to use these membranous "arms" to explore their surroundings, sampling the environment.

"They zip around, all over, until they finally arrive at their target axons. They continue to explore their area and move around and settle into a fairly regular distribution. That's really fascinating to me, and we don't understand it at all."

They also appear to be very flexible,

Kendal Broadie, Ph.D., with an image of an adult fruit fly *(Drosophila)* brain.

ANNE RAYNER



Cross-section through the midbrain of a 4-day-old zebrafish larva. Axons coming from both sides of the forebrain have been labeled with green fluorescent protein, while only those coming from the left have been tagged, in red, with an antibody. Cell nuclei have been stained blue.

Epifluorescence microscopy image by Robert Taylor, graduate student in the Vanderbilt Department of Biological Sciences. Courtesy of Josh Gamse, Ph.D.

he notes. "We're finding that the oligodendrocyte is very plastic … We're beginning to get the sense that there are different kinds of oligodendrocytes. There are certain mutations that result in the absence of one kind of oligodendrocyte, but these may be rapidly replaced by another kind."

After moving his lab to the University of Colorado Denver School of Medicine this summer, Appel plans to continue his search for genes that guide the developmental stages of oligodendrocyte progenitor cells (OPCs) – the immature cells that develop into mature oligodendrocytes – and the genes that determine their unusual behaviors.

To find them, Appel's team uses a traditional genetics approach – causing random mutations in zebrafish embryos and screening these "mutants" to find ones with disrupted oligodendrocyte development.

He hopes that these mutants will point to genes that influence their specification (whether they go on to become an oligodendrocyte or another type of neuron), how fast they divide, and how they recognize and insulate their "target" axon and not other axons.

"We're picking up mutations that affect all of those things," he says. One mutant, called *pescadillo*, or "little fish," produces an excess of OPCs, perhaps due to a genetic defect that causes their multipotent precursor cells (even more primitive cells than the OPCs that can produce oligodendrocytes or motor neurons) to continually divide. Another mutant, which Appel has aptly dubbed *Peter Pan*, has OPCs that "never grow up" – they don't mature into myelinating cells.

His lab will try to identify the genes affected in these mutants – not an easy task, to be sure. But he says, "It's going to be a lot of fun to work through."

Using another approach, a screen for chemicals that disrupt oligodendrocyte development, Appel has found compounds that cause an excess formation of oligodendrocyte lineage cells.

"This was far beyond my wildest dreams because I thought we'd find things that would block oligodendrocyte development," he says. "There are far more ways to block something than promote it."

A chemical that promotes the development of myelin-forming oligodendrocytes may point the way toward therapies for remyelination – which could be beneficial for diseases like multiple sclerosis in which myelin abnormally degrades and results in nervous system dysfunction. Appel is hoping to pursue this lead with a biotech company to determine whether this compound or others like it might be feasible therapeutic targets.

"We need to determine whether this (compound) can direct differentiation of multipotent stem cells into the oligodendrocyte pathway," he says. If so, Appel predicts this compound might become a "super-wonder-drug."

DEATH SIGNAL

One of the more curious aspects of nervous system sculpting is the natural overbuilding that occurs. More neurons are produced than we will ever need or use – and thus are eventually "chipped away." About half of all neurons born will die through a pre-programmed "suicide" mechanism called apoptosis, says Bruce Carter, Ph.D., professor of Biochemistry at Vanderbilt.

"In some places, you lose all the neurons," he explains. "In other places you lose 10 percent. It varies, but about half of the neurons generated die – it's a normal pruning process."

The delicate balance between life and death of brain cells is centered on a family of molecules called neurotrophins. This family includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophins 3 and 4 (NT3 and NT4), and has been an intense area of focus for Carter and colleagues.

The neurotrophins can bind to two different classes of receptors embedded within the neuronal membrane: the Trk family of receptors, which usually promotes survival, and the p75 receptor, which can promote either survival or cell death.

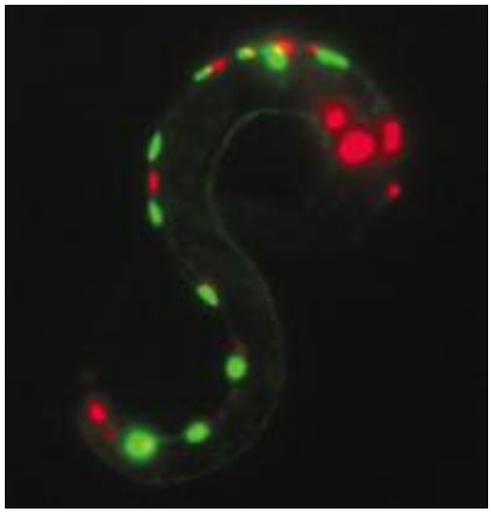
The dual role of p75 had baffled researchers. "It was already known for 50 years that NGF promotes survival," Carter says. "So the idea that somehow the receptor for NGF could cause cell death didn't really make any sense.

"However, what we've learned is that neurons that get the 'right' neurotrophin first will survive through a combined Trk-p75 signal, and they start producing a different neurotrophin, which acts through p75 alone to cause death of their neighbors. Thus, there is a beautifully regulated competition set up so that the proper connections are efficiently established."

How these factors produced such opposing signals was still a mystery. Several years ago, while investigating how these conflicting signals are generated by p75, Carter and colleagues discovered a protein, called NRIF (neurotrophin receptor interacting factor), that binds to part of the receptor and appears to be required for p75-induced cell death. NRIF resembled well-known transcription factors that alter gene expression within the nucleus. The Vanderbilt researchers and others had also determined that NRIF entry into the nucleus induced apoptotic cell death.

"It was kind of puzzling that we found a putative transcription factor 'out there' at the cell surface of the neuron (instead of in the nucleus)," Carter said.

Carter and research instructor Rajappa Kenchappa, Ph.D., have since determined that an enzyme cleaves p75, liberating NRIF from the cell surface and



Motor neuron expressing different colored fluorescent proteins in a *C. elegans* larva.

Image courtesy of David Miller, Ph.D., Vanderbilt Department of Cell & Developmental Biology

allowing it to travel to the nucleus to affect its "pro-death" signal.

This mechanism may explain some of the naturally occurring neuron death during development. Mice lacking p75 have an overabundance of neurons because the cells cannot die, Carter says.

Knowing these 'death signals' could also allow researchers to develop therapies that prevent the undesirable cell death that occurs in neurodegenerative diseases like Alzheimer's as well as after spinal cord injury and stroke.

STRUCTURE VS. FUNCTION

It's not enough just to have the appropriate complement of neurons; they must also connect with other neurons. The formation of these connections, or synapses, sets up communication links between neurons. The ability to alter the strength and number of these connections – a property known as "plasticity" – throughout the entire lifespan of an organism drives behavioral changes and underlies learning.

"Synapse formation … is the end of building structure and the beginning of building function," Broadie says.

In humans, synapse formation begins during late embryonic development (around the beginning of the third trimester) after the bulk of brain "building" is complete. And, unlike the earlier steps of brain development – differentiation, migration and axon guidance to their targets – synapse formation and later plasticity are dependent on neural activity, particularly on sensory activity.

The complex process seems to require an almost inconceivable number of "coincidences."

"You have to have a signaling cell and a receptive cell in register at the same time, the same place, and also of the same 'flavors,'" Broadie notes. These "flavors" are the neurotransmitter systems expressed by the cells. A neuron that produces dopamine, for example, needs to connect with cells that possess a receptor for the neurochemical.

Broadie uses the fruit fly *Drosophila* to dissect all aspects of the life cycle of the synapse: how it's made, how it works, and how it changes throughout the organism's lifespan. One way he does so is in the context of a disease called fragile X syndrome, in which synaptic development and/or function goes awry.

Fragile X disease, the most common inherited form of mental retardation, causes a structural overgrowth of dendrites and axons during development, as well as functional abnormalities in synaptic plasticity later in life.

"There's no question in my mind that fragile X is a disease of development," says Broadie. "But there is a real split in the field whether it is primarily a disease of development, a disease of plasticity, or both."

The answer is vital to developing intervention strategies, Broadie says. "If you want to fix the problem, you absolutely have to know where the problem is – or when the problem is."

Fragile X in humans is caused by altered expression of a gene called *FMR1* (*fragile X mental retardation1*) resulting in the loss of its protein product, FMRP. Broadie and colleagues have developed a *Drosophila* model of the disease and have used the fly model to examine the developmental roles of FMRP.

They've found that FRMP is most highly expressed during a brief window of time during late brain development, and that the protein's expression is increased by sensory input. Their work shows that FMRP plays a critical role in limiting axon and dendrite growth, in particular the activity-dependent "pruning" of neuronal branching, which is vividly illustrated in the overgrowth of neuronal processes and abnormal synapse formation in flies lacking the protein.

"If you compare a fragile X mutant brain to a normal brain, there are fairly severe problems in things like nerve cell structure and synapse formation," Broadie says. "But – and here's the crux of the problem – most of those defects go away." In mouse models of fragile X, he says, after the first month following birth, their brains look fairly normal.

Even though the structural abnormalities appear to go away, the functional problems associated with fragile X persist. Even though the synapses look normal, he notes, it is unclear whether they function properly.

THE DYNAMIC BRAIN

So while the link between altered brain development and the later problems associated with fragile X is being resolved, Broadie and others are already finding factors that might be exploited to improve the symptoms of fragile X.

Because FMRP is a protein that regulates the expression of other proteins, Broadie and his colleagues are looking for genes and proteins that might be affected by FMRP.

Only a handful – about eight – have been proven so far. One protein found by

the Broadie team regulates the internal scaffolding, or cytoskeleton, of neurons, which, he says, "makes perfect sense in that the main defect you see is the change in the structure of nerve cells (and) the cytoskeleton determines the structure."

Another prospect is the involvement of a neurotransmitter receptor called the metabotropic glutamate receptor (mGluR). The receptor – which is activated by glutamate, the main excitatory neurotransmitter in the central nervous system – is important for neuronal plasticity throughout life, and FMRP acts downstream of mGluR activity. Broadie is using the fly model to study the interactions between mGluR and FMRP by manipulating the expression of their corresponding genes in combination.

Studies in mice suggest that excessive signaling through mGluR5 may be responsible for the neurological and psychiatric consequences of fragile X syndrome. Even though FMRP is missing in humans with fragile X, Broadie notes, it may be possible to find ways to manipulate signaling through the mGluR and circumvent some of the later problems of fragile X.

Researchers at Vanderbilt, for example, have identified more than 400 "negative allosteric modulators," compounds that selectively "turn down" the activation of mGluR when glutamate binds to it. With support from Seaside Therapeutics of Cambridge, Mass., they are developing compounds with drug-like properties for further study.

"It's a really innovative idea," says Jeffrey Conn, Ph.D., director of the Vanderbilt Program in Drug Discovery, who is leading the project in collaboration with Craig Lindsley, Ph.D., and David Weaver, Ph.D. "If it works, it could be transformative … It could totally change the way people view developmental disorders."

Unlike sculpture released from stone by the human hand, the brain never achieves a final form. The biological "thinker" is constantly in motion. Throughout life, synapses rearrange and become stronger or weaker, neurons die and (in a few cases) new neurons are born.

Though invisible to the naked eye, this dynamic, continual process of brain sculpting is what gives brain researchers hope that we can find ways to not only treat or prevent diseases like fragile X, but also to just improve the function of the normal brain.

"The brain is not static, it constantly changes itself in response to its environment," says Broadie. "Your heart doesn't do that. Your liver doesn't do that. That's the property that makes it so special.

"That's what makes the brain, the brain." **LENS**

CHOOSING SIDES

The brain carefully conceals its lopsided nature. While it appears outwardly symmetrical, certain functions, like language, are localized preferentially to one side of the brain or the other.

"And because the brain and mind are inextricably linked – the mind is derived from the function of the brain – presumably there are also a number of structural asymmetries as well," says Josh Gamse, Ph.D., assistant professor of Biological Sciences and Cell & Developmental Biology at Vanderbilt.

Gamse is investigating the origins of brain asymmetry in the zebrafish. Because fish don't really have much of a cortex – the part of the human brain involved in "higher" cognitive functions – Gamse is looking at an organ, the parapineal organ, which lies in a more primitive part of the brain, the diencephalon.

The parapineal organ is a cluster of neurons in non-mammalian vertebrates that normally migrates from the center of the brain to the left side and directs asymmetric development of other nearby organs, like the habenular nuclei, which are involved in drinking and feeding behavior, some types of learning and mating.

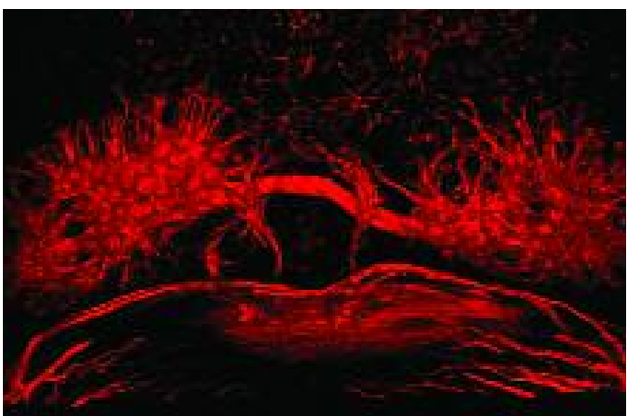
"If you destroy the parapineal early in development, you get symmetric habenular nuclei," Gamse notes. "So there's an instructive role – the parapineal tells the left habenula, 'you're the left habenula.'"

Gamse and colleagues are now screening mutant zebrafish to find those with disruptions in brain asymmetry and identify the genes that cause the abnormal development.

His group recently identified a mutant zebrafish strain, named *from beyond*, in which the parapineal organ is smaller (has fewer cells) and remains in the center of the brain. They've found that this abnormal symmetry is linked to a mutation in the *t-box2b* gene, which encodes a transcription factor expressed in the developing parapineal organ.

Identification of genes that underlie the development of asymmetry in this tiny region of the zebrafish brain may help direct the search for the origins of asymmetry in the human brain. Disorders including autism, dyslexia and schizophrenia have been linked to improper lateralization of the brain, so understanding the biological basis of brain asymmetry may also provide insights into such disorders.

– MELISSA MARINO



The asymmetrical brain

Confocal microscopy image shows a greater density of dendrites in the left habenular nuclei of a 4-day-old zebrafish larva compared to the right. The nuclei are involved in drinking and feeding behavior, some types of learning and mating. Studies of asymmetry – structural differences between the two sides of the brain – may provide insights into human disorders such as autism, dyslexia and schizophrenia.

Image by Robert Taylor, graduate student in the Vanderbilt Department of Biological Sciences. Courtesy of Josh Gamse, Ph.D.


1502

Life in nature makes us recognize the truth of these things, so look at it diligently, follow it, and do not turn away. . . . For, verily, art is embedded in nature; whoever can draw her out, has her.

– ALBRECHT DÜRER

A SHARED PASSION FOR NATURE'S TRUTH

In 1502, German painter Albrecht Dürer turned his realistic style to the rough nap of a young hare's fur and created one of art's masterpieces. Until that time, paintings of animals had lacked dimension, accuracy or understanding of the mechanisms of life that lend the hare its jet whiskers and velvet toes. Dürer, through precise, methodical observation, fused science with art and transformed how man looks at nature.

It's no surprise that Dürer is the favorite artist of developmental biologist Eric Wieschaus, Ph.D., Squibb Professor of Molecular Biology at Princeton University.

BY LYNNE HUTCHISON
PAINTING BY ALBRECHT DÜRER

"I like the technical quality of his work," says Wieschaus, who dreamed of becoming a painter as a boy in Alabama. "I was drawn to developmental biology because it's visual. I had this intuitive understanding of where things are, where they should be. You get that from looking."

Like Dürer, Wieschaus applied his talent for observation and perception to the mysteries of nature – not on young hares, but on Drosophila melanogaster, the common fruit fly.

Using this tiny, hairless insect as a model, Wieschaus was able to identify the genes that determine cell size, shape and position during embryo development. Mutations in these genes alter the fruit fly's normal body plan. These genes later proved to have similar or identical matches in humans, and their discovery has helped transform how scientists look at congenital birth defects.

While Wieschaus' work may never hang in the Louvre, it did bring him the most prestigious award in the world. In 1995, at 48, he received the Nobel Prize in Physiology or Medicine with Edward B. Lewis, Ph.D., of Caltech, and Christiane Nüsslein-Volhard, Ph.D., of the Max Planck Institute for discoveries about the genetic control of early embryonic development.

"The genetic screens carried out by Wieschaus and Nüsslein-Volhard were driven by pure curiosity, but their discoveries had a tremendous impact," says Daniela Drummond-Barbosa, Ph.D., assistant professor of Cell and Developmental Biology at Vanderbilt University Medical Center. "Many of the genes they identified were later implicated in a variety of biological processes with high relevance to human health."

Nüsslein-Volhard, director of the Department of Genetics at the Max Planck Institute for Developmental Biology in Tübingen, Germany, considers Wieschaus the most original scientist – or, perhaps, artist – she has ever met.

"He is singularly prepared to tackle new questions and unconventional approaches," she explains. "He is fun, he is unconventional, and he is charming. Several people completely misjudged his intellect, based on his extremely kind and humble behavior, but he is without doubt one of the smartest people I know. "

Wieschaus met Nüsslein-Volhard at the University of Basel in 1974, while completing his Ph.D. thesis from Yale. The pair discovered a mutual interest in Drosophila embryology and started working together.

Nüsslein-Volhard recalls their times in the lab with fondness. "Eric was loved by the technicians," she says. "Every Sunday he brought a hot meal he had cooked to the lab, walking the 15 minutes through the woods with his big bag. I usually brought in a cake. When we had dull repetitive work to do, we listened to (Mozart's opera) 'The Magic Flute.'"

Wieschaus, who became fluent in German and French during his time in Switzerland, soon left to complete postdoctoral work at the University of Zurich. But he often returned to Basel to finish experiments and plan future studies with Nüsslein-Volhard.

"She was the single most important influence in my work," he recalls. "And she's still a close friend."

In Zurich, Wieschaus began performing experiments on Drosophila with a graduate student named Trudi Schüpbach, who was working on the genetics of sex determination in the fruit fly. After countless late nights in the lab, their scientific collaboration developed into a close friendship – and then into something more.

"It was proximity," Wieschaus explains, his eyes crinkling at the corners as he shares an impish smile with his wife of 25 years. "We started as colleagues."

Wieschaus and Schüpbach married in 1983 after taking faculty positions at Princeton.

Schüpbach, who received her Ph.D. from the University of Zurich, is professor of Molecular Biology at Princeton, where she studies the genetic and molecular mechanisms that cause developmental asymmetries in the Drosophila egg. She and Wieschaus are Howard Hughes Medical Institute investigators and members of the National Academies of Science.

JON ROEMER

Trudi Schüpbach and Eric Wieschaus

OVER THE DINNER TABLE

Perhaps most remarkably, the couple managed to achieve successful scientific careers while raising three daughters. Ingrid, 33, is a lawyer in Boston, Eleanor, 25, a software programmer in New York City, and Laura, 22, a graduate student in social work at New York University.

Eleanor Wieschaus paints a picture of growing up in an extraordinarily stable and loving family, where Schüpbach helped the kids with homework and Wieschaus cooked dinner. "We ate together every evening," she says. "That was non-negotiable, even when I was a teenager and wanted to hang out with my friends. My dad's a great cook – he likes to make Italian.

"It was a great role model to have two parents who loved their work," Eleanor continues. "They showed me that it's possible to have a great career and to have kids who look up to you, respect you and love you." She pauses, then laughs: "But they're just normal people. Well,

semi-normal. After all, they are scientists.

Wieschaus and Schüpbach are characteristically modest about their parental achievements. They point out that juggling a science career and a family can work well – as long as one accepts that the lab and the kids will be the only things in one's life for awhile.

"You get up, get the kids to schools, get to the lab, work all day, then get home and make dinner," say Wieschaus. "Then there are hours of work left to do when the kids have gone to bed. It would be horrible if you didn't love both. If you're happy with just career and family, you'll make it. But if you need anything in your life beyond family and science to make you feel good, it'll be hard."

In the early years, Wieschaus and Schüpbach were too busy raising their girls to discuss research over the dinner table. But now the nest is empty and they talk science a lot more.

"We have side-by-side labs and we share a weekly lab meeting, so we know what's going on with each other," Wieschaus says. "I like the everyday activity that's part of big science more than the great discovery. A lot of scientists have ambition keeping them in the lab. I'm there because I like what I do."

There were few early indicators that either Wieschaus or Schüpbach was destined for scientific greatness. Schüpbach liked math as a girl, but thought it too boring for a career. "I chose science because there were so many open problems," she explains, her voice accented with the lilting charm of Switzerland.

While at university, however, Schüpbach found only two role models for a woman aiming at a career in science. "One woman was in botany and one was

Pictured here: (From top) Trudi and Eric in Olympia, Greece, attending a conference in 1978; at the 1995 Nobel Prize ceremony in Stockholm with daughters Laura (center), Ingrid (behind Laura) and Eleanor (right); Eric at the European Molecular Biology Laboratory in Heidelberg in 1979, where he did his Nobel Prize-winning work; and (bottom, left) with Eleanor and Laura on the beach in 1989 near the Woods Hole Oceanographic Institution in Massachusetts, where Eric and Trudi teach an embryology course most summers. Photos courtesy of Trudi Schüpbach, Ph.D.

in physics," she recalls. "They were workaholics and not married, so they didn't really set an example that a woman could be a scientist and have a family. It was hard then, but it's different now."

Schüpbach has hastened that difference by advising woman graduate students and postdoctoral fellows who want a family and a career in science. "They come and talk to me about when is the best time to have kids, or whether to even have kids," she says.

Role models of a geekier sort influenced Wieschaus' career path. Neither of his parents was a scientist and he had never considered a science career, until he attended a summer science camp at the University of Kansas between his junior and senior years of high school. "It was perfect for a nerdy high school kid like me to hang out with other nerds," he recalls with a laugh.

While a sophomore biology major at Notre Dame, Wieschaus earned much-needed money by washing bottles and fixing fly food in the Drosophila laboratory run by Professor Harvey Bender. There he encountered his first fruit flies and learned basic genetics.

"I like genetics – it's solid," Wieschaus asserts. "You do it and you learn something right there. And Harvey Bender showed me it was possible to have a good life as a scientist. I thought 'Yes! This is life for me.' It wouldn't be weird, it would be perfect."

Wieschaus completed his postdoctoral work in Zurich in 1978, then got his first taste of life as an independent scientist at the European Molecular Biology Laboratory (EMBL) in Heidelberg. Best of all, Nüsslein-Volhard was also working at EMBL. The pair at last could discover how the Drosophila egg developed into a segmented embryo.

TWO SETS OF EYES

Newly laid Drosophila eggs develop in about 10 days, first to larvae, then pupae, then flies. Somewhere in that cycle, certain genes tell each larva to segment into sections that eventually make up the adult fly's head, tail, back and belly. But which of the fly's 20,000 genes controls the process?

Wieschaus and Nüsslein-Volhard decided to look at nature in a different way, just as Dürer had done nearly three centuries earlier. First, they fed the flies toxic substances. This created random mutations that knocked out the function of individual genes. They bred the defective flies, then studied the genetic mutations by peering through a microscope.

For a scientist with an artistic eye, there was much to see. "We sat opposite each other at a dual eyepiece microscope," Wieschaus recalls, smiling at the memory. "We were very competitive. We'd look and one of us would say 'interesting' and the other would say 'not.' I'd say 'mutant.' She'd say 'not.' It helped to have two good sets of eyes. There was a better chance of seeing."

The pair culled through more than half of the 20,000 fly genes, and identified 15 genes in three groups that control embryonic segmentation. The first group of genes, called gap, causes the fly embryo to segment along the head-tail axis. The second group, pair-rule, governs every second segment in the embryo. The third group, segment polarity, refines the individual segments so that the head and tail look different. They published their results in the journal *Nature* in 1980.

"It took us two years to figure out how to do it and one year to do the experiments," Wieschaus recalls. "We knew it was working, but we didn't appreciate what we had done until it was over. We didn't realize the importance until others reacted to it."

Most scientists probably fantasize about receiving the phone call, the one from the Nobel committee, but Wieschaus declares he was not among them when he answered the phone in the fall of 1995.

"It was very early in the morning when I got the call, so I really had no feelings at all," he explains, face deadpan, eyes teasing. "Then I woke up my three daughters, who were mostly interested in going back to sleep."

"I was excited," says Eleanor Wieschaus, who recalls the event with more clarity than her now-famous father. "Princeton is a small town and it was front-page news. I was in eighth grade – the typical attention-seeking middle child. It was fun when the news came out.

"People were saying 'Wow! Your dad won a Nobel Prize!' My dad doesn't come across as the typical scientist. He's brilliant, but to me, he's just my goofy dad."

Schüpbach's happiness over her husband's achievement came with an additional perk – it drew attention to their shared discipline of developmental biology. "I was very proud his work was deemed worthy of the award," she says. "It was very important – not just for Eric but for all the scientists who work in this field."

For Wieschaus, the best part of winning the prize was sharing his parents' happiness and pride. "It meant everything that they could be there in Sweden for the award ceremony," he says. "I couldn't have done it without their support." His mother and late father, who died in 2000, "were very accepting and encouraging of everything I did."

The Nobel Prize award ceremony is a major international event. The Nobel laureates take center stage in Stockholm on Dec. 10, the anniversary of Alfred Nobel's death, when they receive the Nobel Prize Medal, Nobel Prize Diploma and document confirming the Nobel Prize amount from the King of Sweden.

"It was so cold in December, with only four hours of light," Eleanor Wieschaus remembers. "We stayed in a beautiful hotel, and it was the St. Lucia Festival – the festival of light. My sisters and I were so nervous during the ceremony. We were afraid that my dad would fall when he walked up to shake the king's hand. He's a bit of a klutz."

Eleanor enjoyed the limelight in Stockholm, especially when she and her two sisters were interviewed by a Swedish children's TV show. When the host asked if the girls wanted to follow their father's footsteps into science, Eleanor declared, "No – it's too tedious!"

THINK OF GALILEO

After winning the Nobel Prize, Wieschaus spoke at the United Nations and before Congress, then plunged into a river of lectures and appearances that could have inundated a less grounded man. "You become a public person," he explains. "It can get overwhelming. Now

"I like genetics – it's solid," Wieschaus asserts. "You do it and you learn something right there. And Harvey Bender showed me it was possible to have a good life as a scientist. I thought 'Yes! This is life for me.' It wouldn't be weird, it would be perfect."

JON ROEMER

I do a certain amount (of lectures) and no more. The good part is that the attention allows one to be an advocate for science."

Wieschaus and Schüpbach are concerned about the public's perception of science, whether it is human embryonic stem cell research or the theory of natural selection.

"I feel very strongly about this because oogenesis is my field," says Schüpbach. "People don't understand even the basics. When they talk about a certain point when life begins, they don't understand that it's all alive. The egg is alive, the sperm is alive, the mother is alive, the fertilized egg is alive.

"How much do people know about how the embryo is formed?" she continues. "Do people know what a blastocyst is and what it does? People need to know what they're talking about before they start drawing conclusions."

Wieschaus teaches a required undergraduate class for non-science majors at Princeton called "DNA to Human Complexity." His students often challenge him when science conflicts with their religious beliefs, and he has learned to handle the issue philosophically.

"People think their beliefs have a basis in science, but that's not possible," he says. "Your religious beliefs may help you live a moral life, but other people have other beliefs. Science can't say whose beliefs are the best."

Wieschaus tells his students that scientists argue constantly. They inch forward toward some facet of the truth by running experiments and gathering data. But arguing the truth of religion over science doesn't strengthen one's faith.

"You either believe it or you don't," Wieschaus says. "Religious faith is a gift – you can't argue people into it. Religion has always had to deal with new science, and it has always adapted."

"Think of Galileo," Schüpbach interjects. "The Catholic Church fought against his position at the time, but I don't think you'll find a Catholic today who thinks the earth is the center of the universe. I've never understood why the notion of evolution is considered anti-religious. There's no reason why God could not have used evolution."

Wieschaus and Schüpbach also worry about the political manipulation of scientific research.

"There is governmental support for science as an engine that drives military pursuits, but there's no support for science as a whole," Wieschaus asserts. "Scientific facts are being suppressed, especially with global warming. There's no appreciation for the science behind it or the consequences of our actions."

According to Wieschaus, the culprit is not a fear of science, but the belief that science is just another way to make a buck – and that manipulating science can make even more bucks.

"We don't mind different political opinions, but it's emotional for us as scientists," he says. "We see educated people who misuse science, who don't value it. As rare as scientific truth is, it should remain pure."

As pure, perhaps, as an artist's devotion to illuminating the truth behind nature, whether in a fruit fly or a young hare. **LENS**

THE LUB-DUB OF A HEALTHY HEART

Developmental biology guides efforts to "grow" replacement heart valves

The containers on the conference room table are the type you might store leftovers in. But these don't hold last night's spaghetti or week-old casserole from the church potluck. They hold human hearts.

As Joey Barnett, Ph.D., takes the cover off the first container and carefully lifts out the preserved specimen, one man in the group standing around the table steps back and sinks into a nearby chair. The reaction doesn't surprise Barnett, an expert on cardiovascular development at Vanderbilt University; he's well aware that seeing – and handling – human hearts isn't an everyday experience.

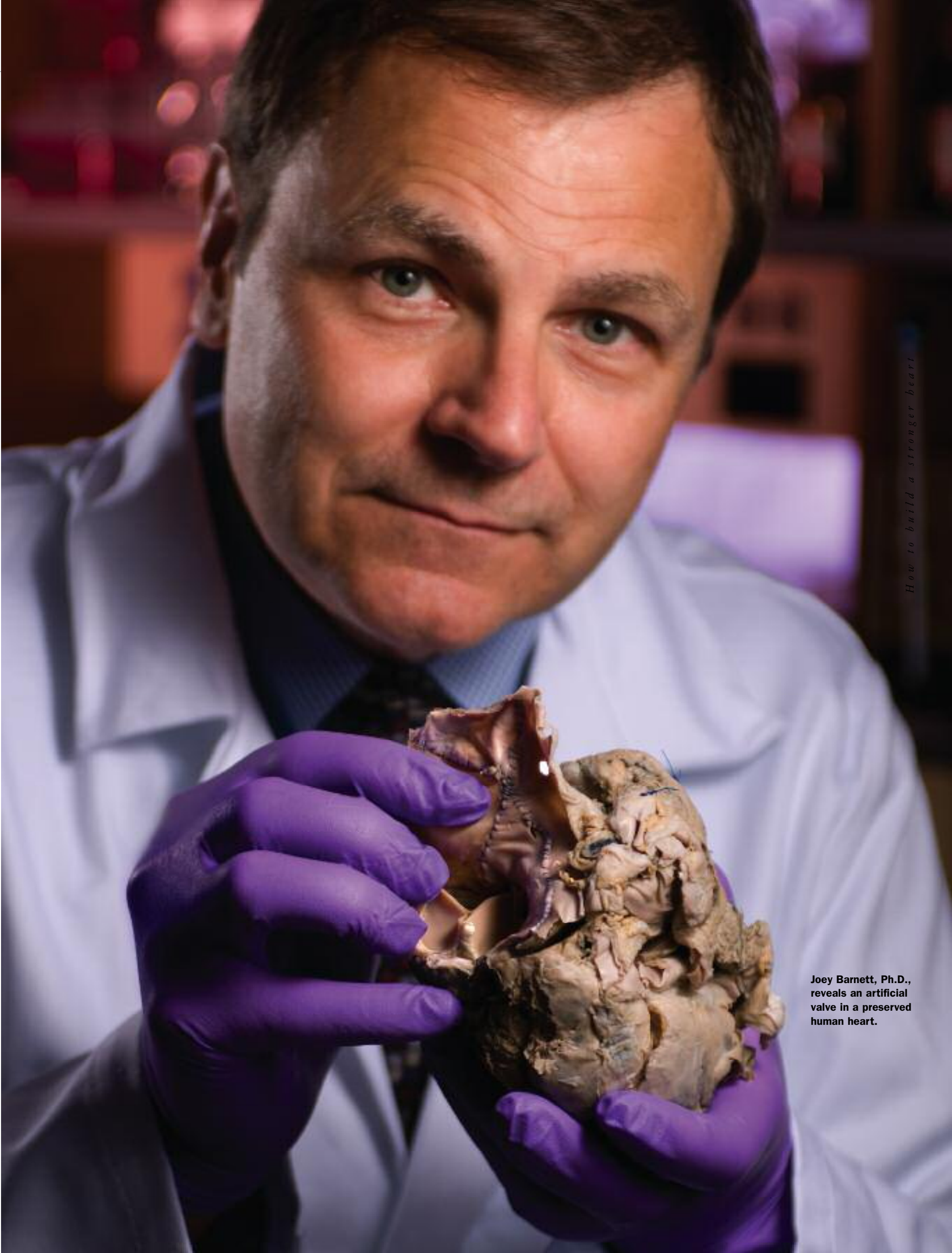
He proceeds to describe how the heart works as a pump, opening it to reveal the heart's right and left chambers, its muscular walls, and the valves that control blood flow. Then Barnett places the first heart into the tentative gloved hands of the man closest to him, to pass around for a closer look.

Barnett reaches for another heart, pointing out the blood vessels and the tough remains of an atherosclerotic plaque – "the most dangerous thing in the western world," he says.

He saves for last the heart that intrigues him most. This heart has been through a lot. The valves at the openings of both the pulmonary artery and the aorta – the large blood vessels that send blood to the lungs and to the rest of the body – have been replaced. The sewn-in aortic valve is a natural tissue valve; the pulmonary valve is artificial.

"This is what we want to do away with," Barnett says, pointing at the artificial valve.

BY LEIGH MACMILLAN // PHOTO BY DEAN DIXON



Joey Barnett, Ph.D., reveals an artificial valve in a preserved human heart.

BY NOW,

the group is eagerly examining the hearts and asking questions – even the man who originally sat down is back on his feet. These on-air radio personalities and producers will be conducting the Children's Miracle Network radiothon in the coming weeks; they are looking at hearts in a Vanderbilt conference room to learn a bit about how biomedical research leads to new therapies.

Barnett, who also is vice chair of Pharmacology, is a good teacher. His hands-on heart tutorial evolves naturally into a discussion of his quest to discover the genes and signaling pathways that build the developing heart and its blood vessels – research that could make it possible to grow replacement heart valves in the laboratory, from a patient's own stem cells.

"Here's the fantasy," Barnett says. "If you need a valve, we take your circulating or bone marrow-derived stem cells, have tissue engineers sculpt the right shape and size matrix, grow the cells on the matrix, and give the complete heart valve to the surgeon.

"Wouldn't that be amazing?"

An outrageous hypothesis

It's the valves in action that give the heart its characteristic lub-dub sound. With each beat, these one-way fibrous doors open and close in a synchronized fashion to keep blood flowing in a forward direction.

The "lub" happens when the mitral and tricuspid valves – the valves separating the atria and ventricles – close. The "dub" corresponds to the closing of the aortic and pulmonary valves.

Valve failure – because of a developmental defect or disease – forces the heart to work harder to compensate for the defective blood flow, leading in many cases to congestive heart failure.

About one in 100 children is born with a congenital heart defect, the leading cause of death in the first year of life.

"Depending on the numbers you use, somewhere between 60 and 80 percent of these kids have an abnormal valve," says Scott Baldwin, M.D., chief of Pediatric Cardiology at Vanderbilt who, with Barnett, is leading the charge to grow replacement heart valves in the lab. Children with valve defects often require multiple valve replacement surgeries as they grow.

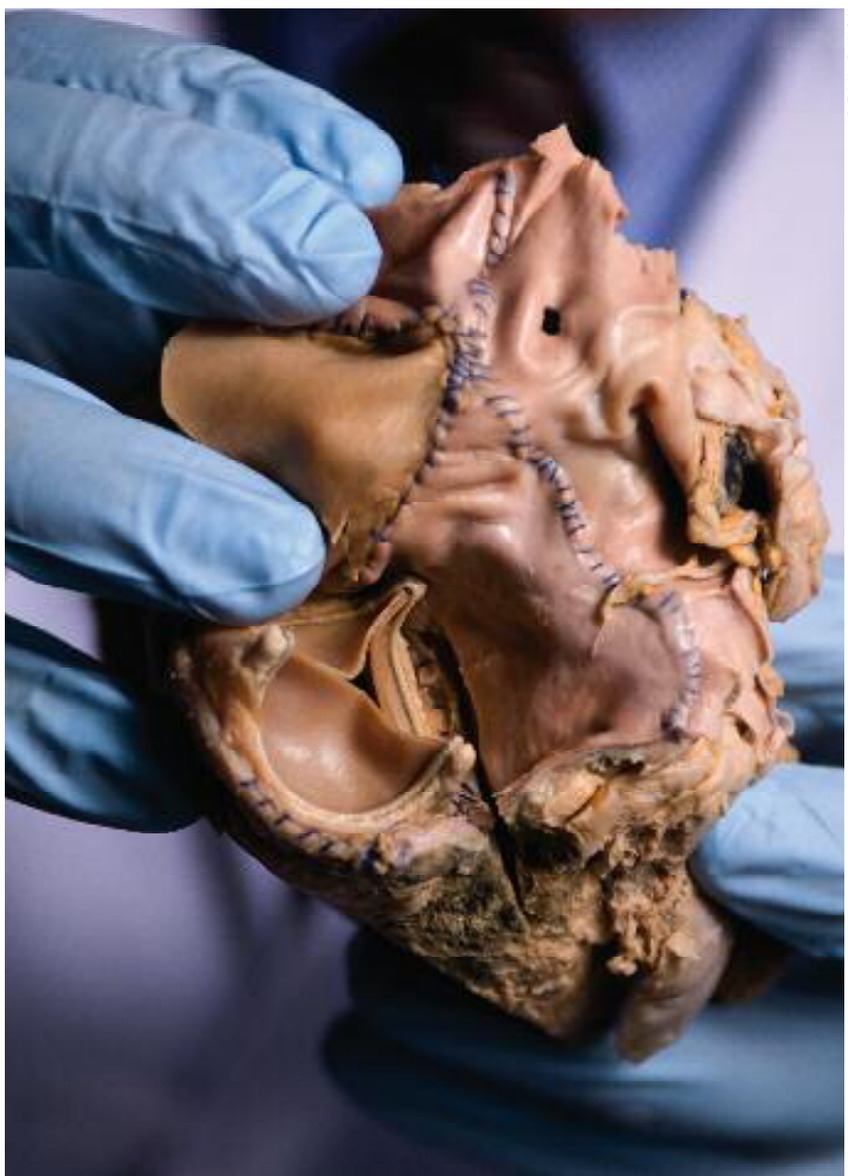
"On the flip side, up to 4 percent of the population over the age of 60 will need to have a valve replaced because it's calcified and thickened," Baldwin says. More than 100,000 valve replacement surgeries are performed each year in the United States, according to the American Heart Association.

"Heart valve problems have become epidemic; it's an important issue," Baldwin says.

So why grow valves in the lab?

Existing options for valve replacements are not ideal, Baldwin and Barnett argue.

Artificial (mechanical) valves are durable, but patients require lifelong blood-thinning therapy. Tissue valves – from pig, cow or human hearts, sometimes with artificial parts – don't usually neces-



DEAN DIXON

STITCHES IN TIME

A preserved human heart (also pictured on page 23) displays its history: a variation of the extensive Ross procedure, in which surgeons replaced a diseased aortic valve with the heart's own pulmonary valve. An artificial valve (shown here) was then stitched in its place.

DEAN DIXON

"We think we've got the building blocks; now we've got to figure out how to put them together."

Scott Baldwin, M.D., holds a key to understanding heart valves – the animal model.

sitate blood-thinning treatment, but they will only last a decade or so.

"In both young and old patients, if we had a durable valve product that doesn't require anti-coagulation or replacement, that would be huge," Barnett says.

Enter SysCODE (Systems-based Consortium for Organ Design and Engineering), an interdisciplinary group that will work toward growing heart valves – and also teeth and pancreatic islets – in the lab.

Led by Richard Maas, M.D., Ph.D., chief of the Division of Genetics at Brigham and Women's Hospital in Boston, SysCODE was awarded a five-year, $24 million grant last year as part of a National Institutes of Health "Roadmap" initiative that is designed to speed the movement of scientific discoveries from the bench to the bedside.

The premise of the SysCODE program, Baldwin explains, is that development follows a "blueprint" for forming complicated organs from a single cell type. It's up to the investigators to decipher this blueprint – determine which genes are the essential ones – and use that information to push the appropriate stem cell populations to form heart valves, teeth or pancreas.

Starting with embryonic stem cells from mice, "we're going to figure out every gene involved in each of those developmental programs," Baldwin says. "Ultimately, I would like to be able to take patients' own stem cells and give them back a valve.

"It's an outrageous hypothesis," he adds, laughing.

The investigators already know a lot about the cells that will multiply, transform and become heart valves. They are a special subset of the endocardial cells that will line the heart.

Let's back up.

When the heart initially develops during the third week of human embryogenesis, it is a simple tube with two epithelial layers of cells: an inner endocardium, which will form the inner lining of the heart, and an outer epimyocardium, which will become the heart muscles that will pump for the lifetime of the individual.

In between the two layers is extracellular matrix, gelatinous material void of cells called "cardiac jelly." At the sites where the heart valves will take shape, Barnett explains, the tube constricts and the cardiac jelly expands, becoming a bulge called the cushion.

"If you look at a heart at this stage of development the bulge is already functioning as a valve," Barnett says. "It's very resilient, so when the heart pumps, the blood moves through and then this bulge snaps back into place to prevent blood from flowing backwards."

Next, a signal (or signals), most likely made by muscle cells, causes some of the endocardial cells to change from epithelial-type cells to connective tissue mesenchymal-type cells, migrate into the cardiac jelly and populate it. This change in cell type, known as "epithelial-mesenchymal transformation" (EMT), is a crucial step in the formation of each of the tissues – heart valves, teeth and pancreas – that the SysCODE consortium will attempt to build in the lab.

Barnett's group and others have extensively studied the EMT process in valve-forming cells in embryonic chick hearts.

His team removes the cushion region from a 2-day-old developing chick embryo, grows it in culture, and adds or subtracts growth factors to evaluate which factors affect cell invasion and proliferation. The group also injects viruses into chick embryo hearts, which are the size of a comma on this page, in order to change gene expression and assess the impact on transformation.

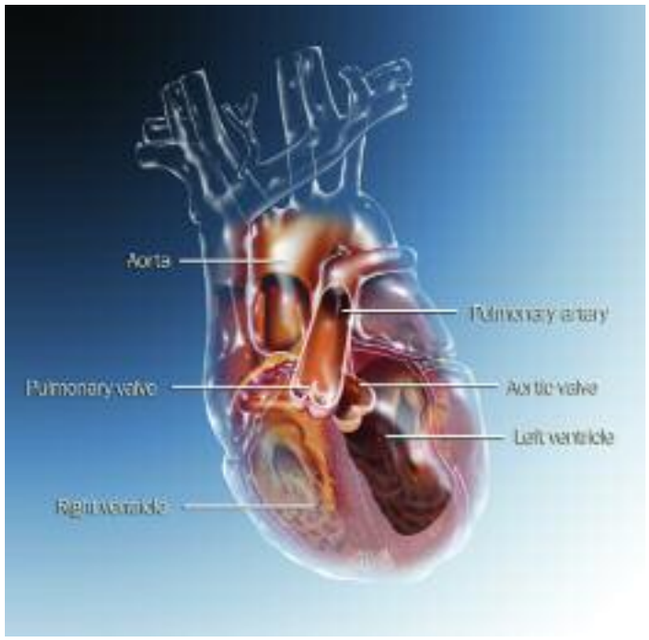
"We know a lot about the transformation process," Barnett says, "but then next we talk about magic happening. You've got the cushion full of cells, and by some combination of genetic and hemodynamic forces, that cushion gets remodeled into what we call a heart valve.

"That's where we're heading with SysCODE, is to understand – and eventually replicate *in vitro* – that remodeling process."

Heart in a Petri dish

Barnett, who earned his Ph.D. in Pharmacology from Vanderbilt in 1986, remembers exactly when he fell in love with the idea of studying the heart. He was visiting Harvard cardiovascular researcher Jonas Galper, M.D., Ph.D., to talk about a postdoctoral fellowship when Galper suggested they take a look at the embryonic chick heart cells the group was just beginning to grow in culture.

"When I saw those beating heart cells,



DOMINIC DOYLE

NATURE'S MARVELOUS PUMP
Cut-away of the heart shows how it works: De-oxygenated blood from the body flows into the right ventricle, which then squeezes it through the pulmonary valve and on to the lungs. Meanwhile, the left ventricle pushes newly oxygenated blood from the lungs through the aortic valve back to the tissues.

I knew immediately that I would be working with Jonas," Barnett recalls. "Here was a cell that was beating in culture, that had a biological response that you could measure – a cell that you could use to really ask questions about how signaling molecules affect and regulate the biology."

Barnett and Galper followed gene expression and protein changes at a particular stage in chick heart development to discover how the heart muscle begins to respond to hormones that regulate heart rate. Before day 3, drugs that normally change the heart rate have no effect; in the next day or so, "those systems come online, and you've got a heart that behaves like an adult heart," Barnett says.

"You can let the embryo tell you: in order to do this biology, these are the tools I need, these are the genes I need to express," Barnett says. "This is one of the great advantages of developmental studies – depending on the model system, you can actually watch the embryos develop and you can see how manipulations alter the biology."

As Barnett was finishing up his postdoctoral studies, a chance elevator conversation with Maas, a friend from graduate school at Vanderbilt, led to a collaboration between the two that kindled Barnett's interest in how the heart's structure forms.

He began to pursue the role of TGF-beta, a widely expressed growth factor involved in cell proliferation and maturation. It was first described in the early 1980s by Mayo Clinic researchers led by Harold Moses, M.D., who later directed the Vanderbilt-Ingram Cancer Center.

TGF-beta also is one of the factors that Barnett and Galper identified as key to turning on the hormone response in chick heart muscle. At Vanderbilt, Barnett and colleagues found that a particular TGF-beta receptor – the type III receptor – is essential for the transformation of endocardial cells in early heart valve formation.

Interestingly, the chromosomal region that is home to the type III TGF-beta receptor gene has been linked to congenital defects in the valves and dividing walls of the heart. Other scientists are looking for the variants that cause the defects.

Barnett and colleagues continue to tease apart the complexities of TGF-beta receptor signaling. They recently discovered that the type III TGF-beta receptor also has a role in the development of coronary blood vessels.

"It's a recurring theme in developmental biology that nature uses the same, or similar, molecules over and over again in slightly different contexts," Barnett says.

Bit of serendipity

Baldwin traces his research path back to a lecture at a Society for Pediatric Research meeting that he attended during his residency. At the meeting, Merton Bernfield, M.D., a Harvard neonatologist and pioneer on studies of the extracellular matrix who died in 2002, talked about how organs take shape.

"I was mesmerized," Baldwin recalls. "I had already committed to a cardiology fellowship, and I remember sitting there thinking 'I want to know how the extracellular matrix influences heart development.'"

During his fellowship research at the University of Iowa, Baldwin published the first paper demonstrating that a component of the extracellular matrix, hyaluronic acid, is important for heart development. And he became intrigued with the question of what patterns the heart – how the single tube loops and twists into an organ with chambers and valves. He suspected that the endocardial lining was involved in laying down the template for the heart.

At the time though – the late '80s – it wasn't possibly to identify endocardial cells, or even their endothelial cell precursors, in the embryo, Baldwin notes. Undeterred, he joined a lab at the Wistar Institute in Philadelphia, where over the next several years he cloned a mouse gene that identified endothelial cells.

Then came a bit of serendipity. In the late 1990s, Harvard immunologist Laurie Glimcher, M.D., knocked out the gene for a transcription factor in mice to study its effect on immunity and found that the mouse embryos died *in utero*. She suspected a heart defect and asked Baldwin to take a look. It turned out that the mice didn't form aortic or pulmonary valves.

Since that discovery, Baldwin and colleagues have found that the gene for this "nuclear factor of activated T cells" (NFATc1) is expressed not only in the subset of endothelium that will become endocardium, but also in the particular endocardial cells that will become the heart valve.

By fusing the gene for a fluorescent protein to the portion of the genome that regulates expression of the NFATc1 gene and then inserting this "marker" into mouse embryos, the investigators can now track – by their glow – cells that are destined to make up the heart valve, isolate them for *in vitro* studies, and even use the system to understand what factors are essential for heart valve formation.

"So we think we've got the building blocks; now we've got to figure out how to

put them together and get them to do what they're supposed to do," Baldwin says.

Part of the "putting them together" means getting the matrix right.

"If you look at a heart valve, it's mostly not cells; it's mostly matrix," Barnett says.

"We know a lot about the cells – we know one when we see one, and we know a lot about the factors that make those cells transform," he continues. "But as far as what's in that matrix, I could fall in a bucket of it tomorrow and not really know what I was in.

"It's the missing component right now."

Richard Caprioli, Ph.D., who directs the Vanderbilt Mass Spectrometry Research Center, will lead efforts by the SysCODE consortium to de-mystify the matrix by identifying the proteins in the cardiac jelly that are important in valve development.

Already, tissue engineers in Boston are making "gel substrates" of hyaluronic acid – the matrix component Baldwin identified as important for heart development.

"As we learn what other components are in the matrix, and how they're organized, our Boston colleagues can make and incorporate those things into the gel," Barnett says, to create the best "scaffolding" for a heart valve.

Ultimately, to grow a valve in the lab may take more than the right scaffolding and the right cells, Barnett and Baldwin acknowledge. The remodeling that takes place after the cells are in the cushion matrix *in vivo* may require pulsatile blood flow, and the investigators are in conversation with the tissue engineers about how to potentially replicate that flow *in vitro*.

The parts will come together, these investigators say.

"It sounds like science fiction, but it will happen in my lifetime, there's no question," Baldwin says. "There's no inherent design limitation; the only thing we don't have is all the information, which is what we need to get."

The investigators are also optimistic that SysCODE's unraveling of the developmental biology "programs" for heart valves, teeth and pancreas will turn up common signaling pathways and explain why it looks like valves turn into bone – become stiff and calcified – when they're diseased.

"We think that maybe when the valve is injured, it reactivates developmental programs to try to repair itself; only now these are not appropriate," Baldwin says. "If we understand the developmental program, I think we're also going to figure out what the pathological program is … and if you can find something to prevent aortic valve calcification as people age, that's going to have a huge world health impact."

The radio folks leaving the conference room seem convinced.

"That was cool," says one. "It's incredible what these researchers can do." **LENS**

GRIDLOCK KEEPS BLOOD FLOWING, HEARTS IN CHECK

Gridlock can be a good thing. If you're talking about cardiovascular development, that is.

In developing zebrafish embryos, a gene and the protein it encodes (both named Gridlock) play key roles in blood vessel formation and heart growth.

Gridlock got its name from what happens when it's *not* working correctly. Blood "traffic" gets snarled in zebrafish with a mutation in the Gridlock gene – the fish fail to develop circulation to the trunk and tail because of a blockage at the base of the aorta.

Tao Zhong, Ph.D., has been tangling with the Gridlock gene since he first isolated it during his postdoctoral fellowship at Harvard Medical School.

Now an assistant professor of Medicine, Pharmacology, and Cell & Developmental Biology at Vanderbilt, Zhong hopes that pursuing the roles of Gridlock and other signaling proteins in heart and blood vessel development will lead to novel strategies for treating human cardiovascular disorders.

In their initial studies of Gridlock, Zhong and colleagues showed that a Gridlock signaling pathway determines whether blood vessel cells will become part of arteries or veins, and that it controls assembly of the aorta.

More recently, Zhong's team demonstrated that Gridlock works with Gata5, another signaling protein that turns genes on and off, to control heart size in developing zebrafish. Gata5 acts like a car's accelerator, turning on genes that increase cell size and division, while Gridlock acts like the brakes, keeping growth from getting out of control.

The findings are exciting, Zhong says, because they suggest that tapping into the Gridlock signaling pathway may offer a way to spur adult cardiac cells to divide.

In damaged adult hearts, cardiac cells increase in size in an attempt to provide more pumping power, but they are not able to divide. Eventually, the individual cells become too large to be effective, contributing to dilated cardiomyopathy (enlarged heart) and heart failure.

"We think that if we can inactivate the Gridlock side of the pathway (take off the brakes), that may provide a therapeutic approach to turn on proliferation machinery in adult cardiac myocytes (muscle cells)," Zhong says.

Zhong is also exploring whether variations in the Gridlock gene may be responsible for some human congenital heart diseases, which occur in one in 100 live births and remain the leading cause of death in the first year of life.

The blood vessel blockage in zebrafish with a mutation in Gridlock resembles a human disorder called coarctation (constriction) of the aorta. Recent studies have also linked a Gridlock mutation to a human septal defect – a hole in the dividing wall of the heart.

Zhong is setting up international collaborations to search for Gridlock and other developmental gene mutations in patients with cardiovascular diseases.

– LEIGH MACMILLAN

NEIL BRAKE

Tao Zhong, Ph.D., peers through a tank of zebrafish.

Q & A

THE SCIENTIST IN SOCIETY

A conversation with S.K. Dey

Sudhansu K. Dey, Ph.D., is the Lova Riekert Chair and Professor of Pediatrics at the University of Cincinnati, and director of the Division of Reproductive Sciences at Cincinnati Children's Hospital Medical Center. The former director of the Division of Reproductive and Developmental Biology at Vanderbilt University Medical Center (he moved to Cincinnati in July 2008), Dey received the 2008 Carl G. Hartman Award from the Society for the Study of Reproduction for his creative and significant contributions to the field.

After receiving his doctorate in physiology from the University of Calcutta, Dey completed his postdoctoral work in reproductive biology at the University of Kansas. He was a member of the faculty there for nearly 30 years before coming to Vanderbilt in 2002. Recently, he shared his thoughts about the importance of developmental biology in understanding human disease, and challenges to the scientific enterprise in the United States.

What do you consider to be your most important scientific contributions to date?

The most significant contribution from our group is the establishment of a novel concept that during early pregnancy, a short delay in the attachment of the embryo to the wall of the womb adversely affects later developmental processes leading to defective feto-placental growth and poor pregnancy outcome.

The state of uterine receptivity, also termed the window of implantation, lasts for a limited period, and it is only during this time that the womb is conducive to support normal embryonic growth. Therefore, the quality of implantation determines the quality of pregnancy and fetal well-being; failure to achieve on-time implantation is a risk factor for an adverse pregnancy outcome.

The birth of this concept is the result of a series of genetic and molecular studies that used genetically engineered mouse models.

Why is developmental biology critical for understanding human disease?

There is emerging evidence for an association between early development and the onset of diseases such as coronary and heart diseases, obesity and diabetes and osteoporosis in adult life. The quality of pregnancy is a critical factor, since subtle changes during *in utero* fetal life can have profound consequences later in life.

Early onset of intrauterine growth restriction, recurrent abortion, preeclampsia (a hypertensive disorder of pregnancy) and preterm delivery are important developmental and reproductive health issues, and are associated with uterine and placental deficiencies. A transient postponement of blastocyst attachment in mice produces detrimental ripple effects throughout pregnancy, indicating that one cause of these end results is defective implantation.

Understanding preimplantation embryo development, implantation of embryos in the uterus, postimplantation embryonic growth and how the placenta forms also will advance our knowledge in several basic physiological processes.

These include: paracrine and juxtacrine epithelial – epithelial interactions and epithelial – mesenchymal-extracellular matrix interactions, involving cell migration and invasion, the formation of blood vessels from bone marrow-derived precursor cells (vasculogenesis) and from pre-existing vessels (angiogenesis), and vascular permeability, as well as regulated growth (proliferation, differentiation, polyploidy and apoptosis).

These processes involve numerous signaling pathways that are common to many other systems under either normal or pathological conditions. For example, many

of the characteristics and signaling pathways that are operative during early development are also active during tumorigenesis – the difference being that tight regulation occurs during pregnancy, while dysregulation of the same pathways occurs in tumorigenesis.

Another interesting area of research is the similarities in plasticity of both multipotent tumor cells and embryonic stem cells (ES). Both these cell types are profoundly influenced by bi-directional microenvironment for expressing specific phenotypes and are amenable to reprogramming. Therefore, understanding the intricacies of early development might help to better understand the complexities of tumorigenesis, and might one day reveal that "life and death are linked by a common thread."

What are some of the challenges to making further progress?

The entire research enterprise in the United States is at a crossroad.

On the one hand, enormous technological advances have set the stage for ground-breaking discoveries, but on the other hand, dwindling federal research dollars for basic research make it difficult for scientists to take advantage of this opportunity.

In addition, federal, state and institutional bureaucratic regulatory burdens (for example, compliance with animal protocols and institutional review boards) are creating a tremendous stress on investigators and raising the levels of despair and frustration in them, resulting in reduced scientific innovation and productivity. Investigators are spending more and more time in writing and rewriting grants and addressing and untangling bureaucratic red tape.

Like adding salt to the injury, increases in research costs are passed on to investigators by institutional leadership in the face of shrinking federal research dollars provided through the National Institutes of Health (NIH). Investigators, especially junior and mid-career scientists, are increasingly worried that they will be unable to put bread and butter on the table for their families if they fail to receive grants that provide a major portion of their salaries.

These are not the only challenges the scientists are now facing. Federal restrictions on human stem cell research in the United States are also hindering progress in a field that has enormous clinical applications in regenerative medicine and correcting genetic errors that lead to various diseases.

Scientists here and in Japan have circumvented some of these obstacles by creating induced pluripotent stem (iPS) cells in mice and humans through the ectopic expression of four transcription factor genes (Oct4, Sox-2, Klf4 and Myc) in non-embryonic cells. While this is a huge breakthrough in the field, iPS cells must be compared alongside with human embryonic stem cells to determine their utility as true pluripotent cells.

The unwillingness of the U.S. government to allow the expansion of the repertoire of human stem cell lines is having dire consequences on stem cell research and it is driving prominent scientists to pursue their work outside the country.

There is a move by the Center for Scientific Review at the NIH to reorganize the peer review process to reduce the length of grant applications, to ensure high-quality review by experienced reviewers and a quick turn-around time of reviews for new investigators, and to provide an open deadline for submitting

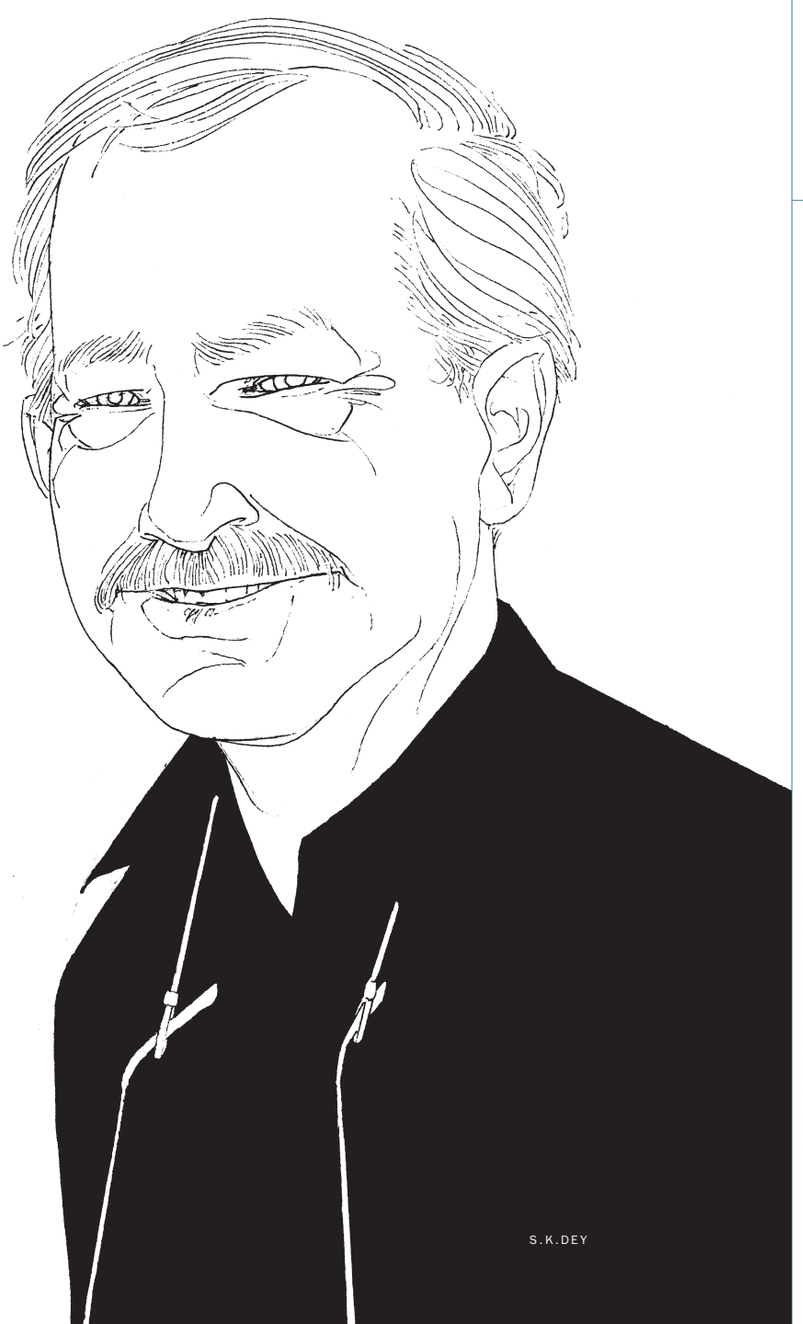


ILLUSTRATION BY DAVID JOHNSON

grants by reviewers. All of the changes that are being implemented or planned to be implemented have good intentions.

While some of the changes will be welcomed by the investigators, if the funding situation does not significantly improve, we scientists can be listed as an endangered species. This is a very difficult time for the entire research enterprise in the United States, and we – meaning the government, general public, scientists and their institutional leadership – must work together to address these issues.

What is the responsibility of the scientist to speak up, to challenge government policies and society itself?

The scientific community should forcefully articulate the problems to the leadership at the institutional, state and federal levels without any reservation. The scientific societies should follow the same suit which they do by lobbying to Washington. These are good practices, but often do not meet with success.

What we need is a "million scientists march" to Washington involving scientists, educators, graduate students, postdoctoral fellows, research personnel, people from biotech and pharmaceutical companies, and citizens who care for scientific discoveries that improve health and mankind. This approach may educate the society at large, draw the attention of decision-making bodies and raise the stature of scientific research and the benefits society reaps from it.

The NIH Roadmap and its emphasis on big science and translational research are good concepts, but these concepts should only be pursued if Washington appropriates separate funding to NIH for these purposes, not at the cost of investigator initiated basic science research projects. Otherwise, we may lose a generation of young and mid-career investigators.

As Judith Bond, Ph.D., former president of the American Society for Biochemistry and Molecular Biology, wrote in 2006: "Funding strategies must provide opportunities for exploring new ideas, taking advantage of an unexpected finding or serendipitous discovery. There is no single path to discovery, problem-solving and knowledge creation."

Is the preeminence of U.S. science being threatened by the "globalization" of biomedical research?

Surely, the current bureaucratic regulatory burdens and dwindling funding environment in the United States have created a great deal of anxiety in the scientific community. Our preeminence in scientific leadership is being threatened by increasing research investments in Europe, Japan, China, India, Singapore and South Korea. This rise in research growth in other countries will boost the U.S. scientific enterprise only if we embrace and partner with them from our strengths, not from our weaknesses.

If we increase our investments in science and take our research to a new level, then scientific interactions and exchanges will bring benefits globally to humankind. In failing to do so, we will face a reverse brain drain, meaning that U.S. scientists will relocate their research programs in those countries.

This has already started. Several U.S. scientists have relocated their programs in other countries, and many foreign-born scientists who settled in the United States for the quest of science are now returning to their home countries to further their scientific pursuits. The scientific environment here is becoming less attractive to them. The situation is likely to get worse, since fewer U.S. students are interested in pursuing a science career. There should be an all-out effort at the national and local levels to combat this deteriorating situation.

What must we do to protect and nurture quality science in this country?

If we want to maintain our leadership position in science and technology, there has to be a radical change in our culture at all levels. There has to be an infusion of resources for pursuing careers in science and to convince our young generation that pursuit of science is noble and serves humankind.

We need to see substantial increases in federal funding to stop further erosion within the scientific community. There are now remarkable opportunities to establish scientific exchange programs with other countries which are substantially investing on science and technology.

Does the United States have a responsibility to aid the scientific enterprise in developing countries?

Absolutely. One major objective of scientific discoveries is to fulfill human needs and curiosity. Everyone in the world should have that privilege and opportunity, especially in these days of globalization. The only way this objective can be fully realized is if the developing countries also engage in scientific pursuits, but they will, of course, require help from other advanced countries.

Where will we be in 10 or 20 years in our ability to understand human health, and intervene to treat or prevent disease?

My guess is that molecular and personalized medicine will take center stage. There will be significant advances in our understanding of the genetic and epigenetic causes of human diseases, such as cancer, Alzheimer's, Parkinson's, diabetes, obesity and infectious diseases like HIV. The success of these research initiatives will, however, depend upon the national investment in basic sciences.

What advice do you have for the young person who is considering a career in science?

Have dreams and passion for knowing the unknown. It should be made clear that doing research is not a glamorous profession or hobby, it is a passion.

Upon taking office as president of the American Society for Cell Biology in 1997, Mina Bissell, Ph.D., said, "If biomedical research is truly what you want to do, then you must be willing to pay the price ... It takes time, patience, stubbornness, years and years of seven-day weeks and 18-hour days, years of poverty-level wages, predictions of doom and failure, rejections of papers and grants, depression and self-doubt ... But one persists. One continues because this is what one must do. This is what you want to do."

The passion for research needs to be seeded when students enter high school and college. It should be made clear that the pursuit of scientific research is only for those who are truly dedicated to this endeavor. The students should be reminded that the pursuit of science is a wonderful world if you love it.

Of course, there must be in place the resources and infrastructure to nurture the dreams, imagination and passion in young men and women who are considering careers in science. **LENS**

Riding the neural crest

Studies that track cell migration, fate illuminate gut disorders

BY LEIGH MACMILLAN

During the fourth week of human embryonic development, in the ridges of the closing neural tube, a remarkable group of cells emerges.

Named for their birthplace, these "neural crest" cells journey to sites near and far in the developing embryo, where they form a wide array of tissues, including the peripheral nervous system, facial skeleton and melanocytes in the skin.

The fate of an individual neural crest cell – what it becomes – relates to both its starting position (top-to-bottom) along the neural tube and to its migration path, explains Michelle Southard-Smith, Ph.D.

She's interested in the cells that trek from the neural tube ridges into the future gut and along the length of the developing intestine. There, they form the neurons and glia of the enteric nervous system – the "brain" of the gut that controls motility, mucosal transport, tissue defense and vascular perfusion of the gastrointestinal tract.

"These cells have the longest neural crest migration that occurs in the developing embryo," says Southard-Smith, assistant professor of Medicine and Cell & Developmental Biology at Vanderbilt. "Variations that impair the ability of those neural crest cells to complete the migration or to survive and become functional neurons and glia in the gut wall can cause gastrointestinal disorders like Hirschsprung's disease."

Patients with Hirschsprung's disease are missing enteric ganglia (nerve bundles) in the intestine, causing constipation and blockages and requiring surgical intervention. Hirschsprung's occurs in one out of every 5,000 live births in the United States and can be fatal.

The severity of the disease depends on how much of the large intestine is affected – how successful, or not, the neural crest cells were in migrating through and populating the gut, Southard-Smith explains.

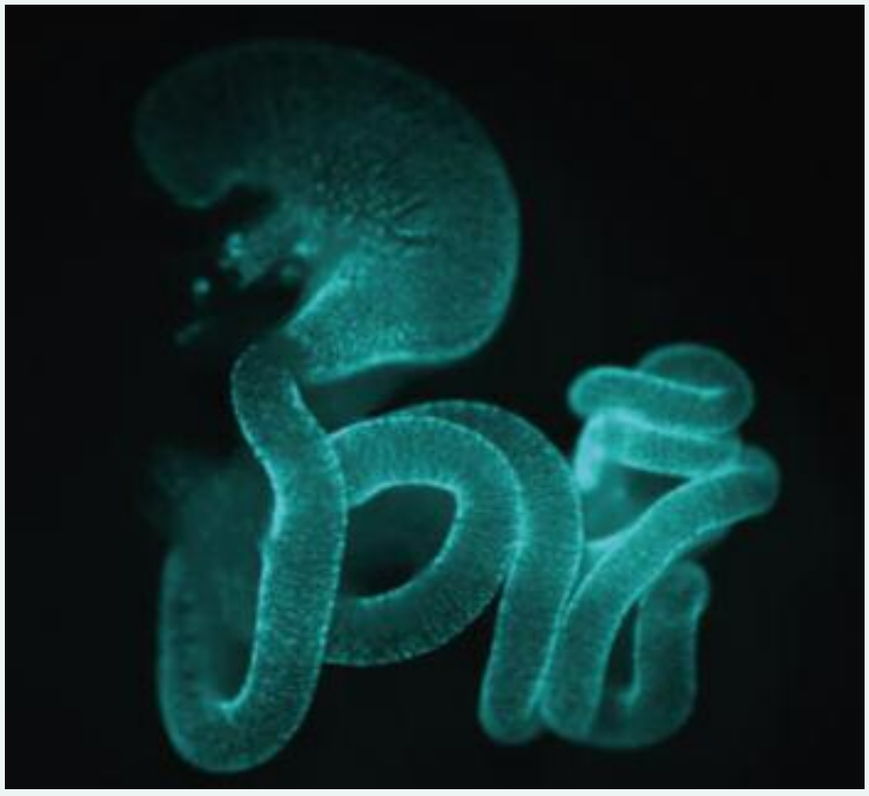
To explore how neural crest cells migrate and make decisions about their fate, Southard-Smith and colleagues have developed genetically engineered mice using two genes important for enteric nervous system development, Sox10 and Phox2b, to drive the expression of fluorescent proteins. In these mice, the neural crest cells that populate the enteric nervous system "glow" a vibrant blue-green.

Following the glowing cells with imaging technologies has already revealed a surprise: differences between neurons and glia are evident when the neural crest cells are just starting their journey to the gut, suggesting that cells make fate decisions earlier than scientists believed, Southard-Smith says.

In addition to tracking cells as they migrate, the researchers are capturing the glowing cells and culturing them in the laboratory. The aim is to understand how the cells respond to various growth factors and to evaluate their ability to form enteric nerves and glia after transplantation into a mouse model of Hirschsprung's disease.

Ultimately, the research could offer treatment options for patients with the disease, Southard-Smith says.

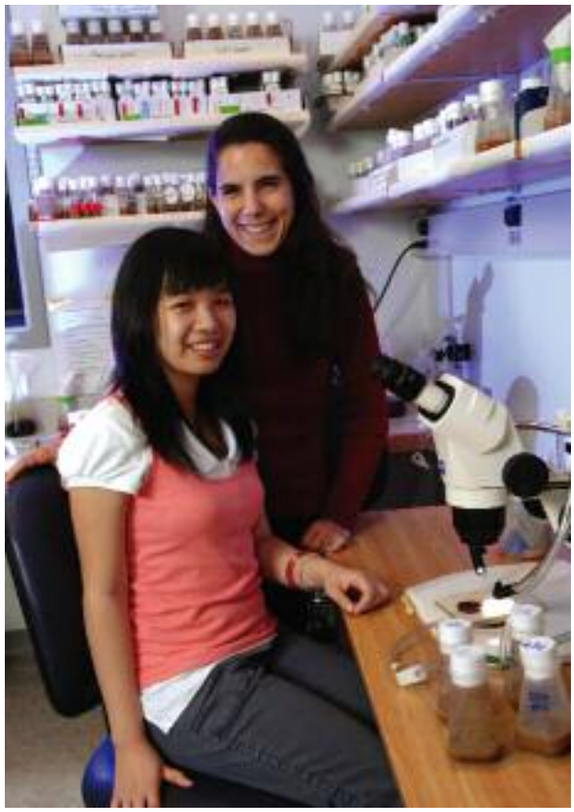
"There are neural crest cells in skin – we're hoping to take them out and reprogram them with cues to make them become enteric neurons and glia," she says. "That's where we're going with this." LENS



The migration of neural crest-derived cells in the developing gut is revealed in a transgenic mouse embryo by the Cerulean Fluorescent Protein, a marker of Phox2b expression. The transcription factor Phox2b guides development of the enteric nervous system, which controls motility and other gut functions. By tracking migration of early nerve progenitors that express Phox2b, scientists hope to identify, and eventually correct, abnormalities that can cause gastrointestinal disorders in humans.

Confocal microscopy image by Vanderbilt research assistant Ashley Cantrell. Courtesy of Michelle Southard-Smith, Ph.D.

Reprinted from Developmental Dynamics, Vol. 237, No. 4, 2008, pages 1119-1132.



ANNE RAYNER

Uyen Pham (seated) with her mentor, Daniela Drummond-Barbosa, Ph.D.

The next generation

By Lillian Gu

Uyen Pham spent part of her summer vacation in 2007 counting fruit fly eggs.

The high school senior participated in a research study at Vanderbilt University Medical Center to better understand the link between nutrition and fertility.

"I liked counting the eggs," said Pham, who graduated in the spring of 2008 from Hillwood High School in Nashville. "It was very relaxing."

Pham was a member of the inaugural class of seniors in the School for Science and Math at Vanderbilt, a research-centered learning experience offered to high school students in Metropolitan Nashville Public Schools by the Vanderbilt Center of Science Outreach (www.scienceoutreach.org).

During the school year, students receive college-level instruction and participate in research at Vanderbilt one day a week, while keeping up with their regular high school classes.

"One of the primary goals of this center is to connect university scientists and K-12 education," explained center director Virginia Shepherd, Ph.D., professor of Pathology. "The school is a unique model of how that can be accomplished."

Pham moved to the United States with her family from Vietnam six years ago. "Like all immigrants, we were looking for a better life," she said. "My parents were hoping that my sister and I would receive better education ... and we did."

When she was in ninth grade, a dedicated biology teacher, Cathy Morgan, inspired Pham with her hands-on approach to learning. Pham was fascinated with DNA extraction and other techniques, and was immediately interested when she came across the School for Science and Math while surfing the Internet for summer internship opportunities.

"I like the fact that science is always changing and growing," commented Pham. "It's intriguing and fun!"

Pham requested the field of developmental biology when she applied, and was pleased to be placed in the lab of Daniela Drummond-Barbosa, Ph.D., assistant professor of Cell and Developmental Biology.

It is well known that poor diet negatively affects fertility. In the fruit fly, *Drosophila*, a protein-poor diet causes egg production to drop. The Drummond-Barbosa laboratory has shown that this ovarian response to diet involves the insulin signaling pathway.

Under the guidance of postdoctoral fellow Hwei-Jan Hsu, Ph.D., Pham studied a family of transcription factors called FOXO. These proteins regulate insulin's effect on cell growth by turning genes on and off, but their effect on egg production is largely unknown.

During her seven-week-long research project, Pham counted eggs produced by normal flies when they were given protein-rich and protein-poor diets, and eggs produced by mutant flies, in which the genes for the transcription factors had been "knocked out."

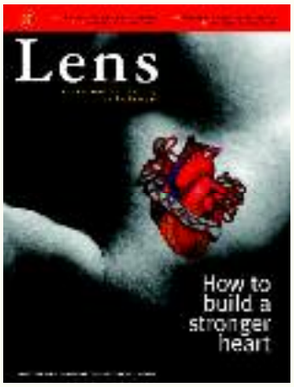
Normally, flies lay many eggs on a rich diet and only a few on a poor diet. If FOXO were required to repress egg production on a poor diet, Pham hypothesized, the mutants without FOXO should not respond to dietary changes.

However, she found that the mutants did, in fact, produce fewer eggs when given a poor diet, indicating that FOXO is not necessary for the response of the fruit fly ovary to diet.

For her research project, Pham was recognized last fall as one of seven Tennessee semifinalists in the prestigious Siemens (formerly Westinghouse) Competition in Math, Science and Technology.

"Uyen is participating in research which is often reserved for undergraduate and graduate students," said Glenn McCombs, Ph.D., director of the School for Science and Math. "She exemplifies what we envisioned would be possible for students attending the school."

Accepted into Vanderbilt's class of 2012, Pham wants to major in biology, and is contemplating a career in developmental biology. **LENS**



To the Editor:

As always, you have published another excellent issue of *Lens* with the picture on the cover and its title, "How to Build a Stronger Heart."

Prior to opening it, I thought that the issue would cover the non-technical, non-pharmaceutical means of achieving heart-health. On pages 25-27 of your excellent article, you seemed to be getting close, but you never quite made it. Vanderbilt remains a high tech place and will mostly likely remain so.

I would like to make a slight criticism of the box entitled, "The importance of knowing your numbers" (page 27). You missed a real opportunity to educate your readers.

Although I am sure that they are well educated scientists, far and away the majority of physicians with whom I have discussed their own "numbers" don't know which numbers to measure. Dr. Potts was quoted, "Know your numbers – blood pressure, cholesterol level, weight and blood glucose." More specifically, he should have said, "Blood pressure, waist circumference, triglyceride/HDL ratio and HgbA1C."

Patients understand with a little explanation. And, the more information we can give them, the better they will do.

RICHARD C. ADLER, M.D.
Alumnus, Vanderbilt University
(B.A., 1955; M.D., 1959)
Clinical Assistant Professor of Preventive Medicine, University of Tennessee School of Medicine
Medical Director, Alpha Maxx Healthcare, Inc.

Erratum

An illustration of ion channels by J.P. Cartailler, which appeared on page 14 of the last issue, "How to build a stronger heart," should have included the following information: © 2007 by Symmation LLC

Letters to the editor may be e-mailed to: lens@vanderbilt.edu; Faxed to (615) 343-3890; or mailed to Editor Bill Snyder c/o Vanderbilt University Medical Center D-3237A Medical Center North Nashville, TN 37232-2390

now on the web

Check out the new Lens Web site at www.mc.vanderbilt.edu/lens or www.lookatscience.org

Footprint on the moon

This photo taken by Apollo 11 astronaut Buzz Aldrin is now an iconic image of the first manned mission to land on the moon on July 20, 1969.

IN THE NEXT ISSUE:

Science's uncertain footing
Beset by budget cutbacks and a skeptical public, biomedical research enters the lean years.

Eating the seed corn
A research "brain drain" imperils the nation's security and prosperity, and the search for cures.

What to do?
Innovation and leadership in the private sector – and in other countries – may point the way.

Lens

Vanderbilt University
VUMC News and Public Affairs
D-3237A Medical Center North
Nashville, TN 37232-2390

PRSRT STD
U.S. POSTAGE
PAID
NASHVILLE TN
PERMIT NO.
777

Vanderbilt Medical Center