

MUSCLE CONTROL

A cross section of an embryonic chick's spinal cord shows the nerve pathways taken by a chick's own motor neurons (red) and by motor neurons derived from mouse embryonic stem cells (green). The transplanted mouse cells performed as well as the chick's own cells.



Healing Connections

A handful of researchers are making progress with mouse embryonic stem cells—connecting nerves to muscles and improving mobility in animals. **BY MAYA PINES**

Stem cells—the precious and finicky immature cells that can grow into specialized cells or renew themselves—are finally being harnessed. After years of research into how organisms develop from a fertilized egg into an adult and how the earliest cells differentiate, a few teams of scientists are finding ways to make stem cells do their bidding, at least in mice and other animals.

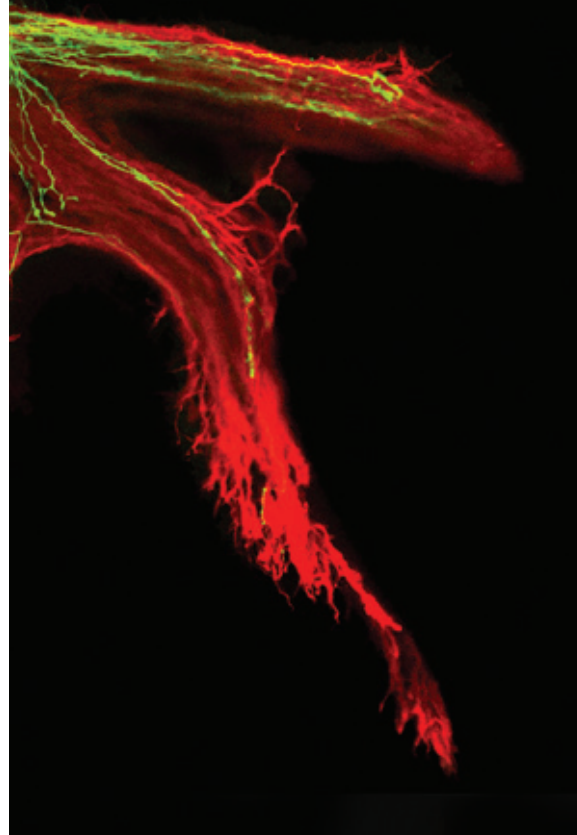
One team of scientists has coaxed mouse embryonic stem (ES) cells to grow into motor neurons—the specialized nerve cells that control the movement of muscles. When the team inserted these newly made motor neurons into a chick embryo's spinal cord, they found that the neurons grew long extensions called axons and made contact with muscle cells just as well as the embryo's own motor neurons did. This extraordinary finding by HHMI investigator Thomas M. Jessell at Columbia University's College of Physicians and Surgeons and his colleagues was given early publication online by *Cell* on July 17, 2002, and was published in the August 9 issue. It raised hopes that several incurable muscle diseases, including amyotrophic lateral sclerosis (ALS, also known as Lou Gehrig's disease), as well as spinal cord injuries, might eventually be treated with such cells.

Along similar lines, Ron McKay and his team at the National Institute of Neurological Disorders and Stroke in Bethesda, Maryland, have successfully directed mouse ES cells to become neurons that release dopamine, the chemical lacking in Parkinson's disease. The scientists also showed that these neurons restore some aspects of mobility in a rat model of Parkinson's, a possible harbinger of more effective therapies for this common disease.

All stem cells are immature, unspecialized cells with great potential, but some are more limited than others. ES cells—those derived from very early embryos—can become any type of cell in an organism and can renew themselves indefinitely. But adult stem cells have already started on a particular pathway of differentiation. They can still generate a variety of cell types, but the choices are restricted.

HHMI investigators Allan C. Spradling, Sean J. Morrison and Mark T. Keating (see sidebar, page 26) and others are examining how adult stem cells might be made to treat diseases. The therapeutic potential of adult stem cells is particularly important because of current U.S. restrictions on research involving human ES cells.

THE RIGHT SIGNALS For his systematic, decade-long study of how the nervous system functions—“specifically, how different cell types in the vertebrate nervous system actually become different”—Jessell relied initially on stem cells from embryonic neural tissue. At first, he could not even tell these neural progenitor cells apart. “At the very early stages of development, it's impossible to recognize a motor neuron or a sensory neuron by its appearance,” he explains. But each class of neurons turns on a different set of genes. These genes are activated by different sets of transcription factors, proteins whose signals turn on specific



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genes in a cell's nucleus. Because these factors could be recognized by specific antibodies that were available to him, Jessell soon learned to identify various types of neurons at their earliest stages.

While studying the early developmental signals that normally produce motor neurons, Jessell decided to find out whether “naïve ES cells” could be converted into motor neurons by means of the same signals. With great satisfaction, he now reports that the answer is yes—at least in mice. Hynek Wichterle, a Czech postdoctoral fellow in Jessell's lab, recently proved it in an experiment that “demonstrates one can learn from embryonic development and apply it directly to ES cells. Just by exposing the mouse ES cells to developmentally relevant signals, we drove them to differentiate all the way to motor neurons.”

Wichterle notes that he had “a huge chunk of luck”: He managed to produce all these changes by using only two signals. One is retinoic acid, a product of vitamin A that binds to receptors in the nucleus. The other is Sonic hedgehog, the product of a gene that was first identified in developing fruit flies and that Jessell found expressed in the mouse spinal cord, where it plays a critical role in the differentiation of motor neurons.

“There are three main steps that an ES cell needs to take in order to become a motor neuron,” says Jessell. “The first is the ‘decision’ to

become a generic neural progenitor cell. The second is the decision to become a spinal cord progenitor cell. The third is the decision to become a particular progenitor cell that gives rise to a motor neuron.” The retinoids “are good at converting neural progenitor cells into spinal progenitor cells, and then hedgehog is good at converting spinal progenitor cells into the kind of cells that are precursors of the motor neurons,” Jessell says. “Where we got lucky is with the first step. For some reason, ES cells tend to become nerve cells almost by default, as first suggested by HHMI investigator Douglas Melton (at Harvard University).”

The third step was not so simple, however, because different concentrations, or grades, of sonic hedgehog have different effects. “Hedgehog's graded actions normally produce at least five different classes of neurons,” Jessell points out. “Typically, only 25–30 percent of the cells that emerge from exposure to sonic hedgehog are motor neurons. I think it's going to be essentially impossible, in the context of neural stem cells, ever to find situations where 100 percent of the generated cells are of one particular neuronal subtype.”

To get around this problem, he explains, “we used a genetic trick. Wichterle, together with postdoctoral fellow Ivo Lieberam, marked the motor neurons with green fluorescent protein. Then by putting all the cells through a fluorescence-activated cell sorter, we could separate the fluorescent ones from the others and get essentially 100 percent purity.”

DIRECTIVE SIGNALS Thomas Jessell can turn mouse ES cells into motor neurons.

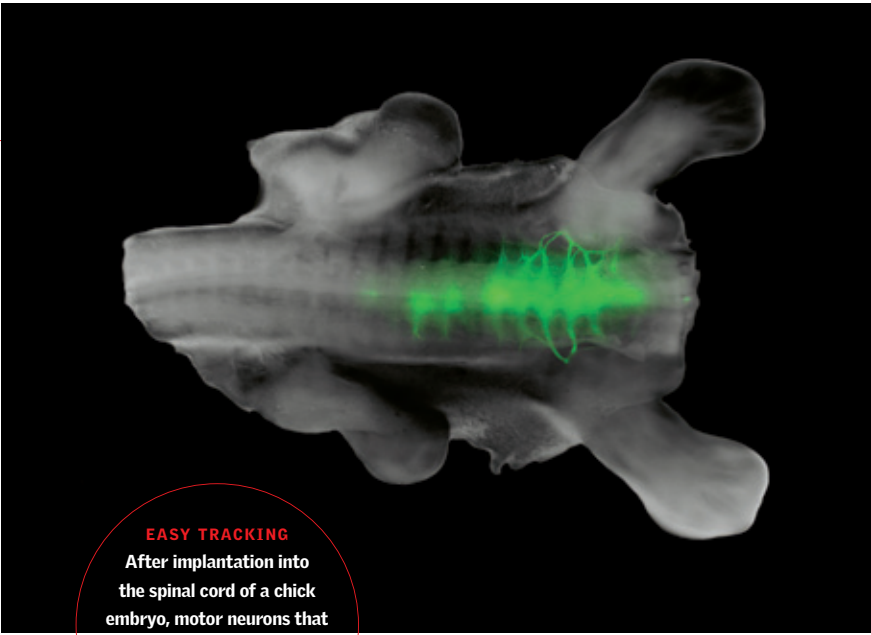


MARC BRYAN-BROWN

RATIONAL DESIGN Ultimately, Jessell is seeking precision, control. He points to several clinical trials in which people with Parkinson's disease were treated with human cells that produce dopamine, “not of ES cell origin but of fetal brain origin.” A few patients benefited from these attempts, but some actually got worse. “That may be because the cells introduced into those patients were a heterogeneous mixture,” Jessell says. “It's not surprising that the clinical outcome is variable if you cannot control the precise number or proportion of dopamine-producing cells that you are putting in.”

Such control would be even more important in dealing with other diseases, he believes. “In Parkinson's, at least you know that you really need dopamine-producing neurons,” he says. “But in other diseases, it is not clear which cell type is needed to reconstruct a circuit or prevent neural degeneration. In the context of ALS, for instance, do you want to put in motor neurons or would you be better off with spinal interneurons or glial cells, which normally surround the motor neurons and may actually support the survival of the remaining ones?” Or, for that matter, should all classes of spinal cord cells be used?

Having a way to purify the appropriate cells “puts you in a position now where you can ask such questions,” Jessell says. He is also pleased that “we have manipulated mouse ES cells simply by exposing them to different environmental signals—we have not changed the genetic makeup of the ES cell itself,” he says. While some other scientists introduce genes directly into the ES cell, “we have just added factors that we know are involved in the normal developmental process and let the ES cell do the rest.” As a result, Jessell has gained a tool



EASY TRACKING
 After implantation into the spinal cord of a chick embryo, motor neurons that were derived from mouse ES cells can be recognized by their green fluorescence.

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Massachusetts, has identified a small molecule that activates the hedgehog pathway, so we can now direct ES cells to become motor neurons simply with small, synthetic chemicals. You don't even need mystery factors anymore."

Eager to test whether the neurons they grew from mouse ES cells were functional motor neurons, Wichterle decided to put them into live chick embryos. Why chicks? "Because their embryos grow in eggs rather than wombs," Jessell explains. This makes it much easier to gain access to the neural tube and "introduce the ES cell-derived motor neurons into the spinal cord at exactly the same time that the host motor neurons are being generated." In a sense, Jessell says, "we are giving the ES cell-derived motor neuron an even chance."

The scientists found that "even in this completely different host species, the ES cell-derived motor neurons settle in the right place in the spinal cord, extend axons out and form differentiated synapses with target muscle" over the same time course as the chick's own motor neurons. In fact, Jessell says, "it was a dead heat." The fluorescent green markers continued to function, so he could follow the path of the ES cell-derived motor neurons.

His team has already started to explore whether human ES cells will behave in the same way. "We are collaborating with a group of

that might eventually be used with human ES cells as well. "If we wanted to apply this strategy now to a human ES cell, we could simply take the same factors," Jessell says. "It turns out that the two factors we use—Sonic hedgehog and retinoic acid—cross species barriers. Furthermore, one of our collaborators, Jeff Porter at Curis, in Cambridge,

Discovering the Genes for "Stemness"

Douglas A. Melton, an HHMI investigator at Harvard University, is exploring what gives stem cells their special abilities—what accounts for their "stemness." And he has found hundreds of genes likely to play a role.

Together with his colleague Richard C. Mulligan and other Harvard associates, Melton compared the activity of the three best-known types of stem cells—embryonic, neural and hematopoietic (blood-forming)—with the activity of other types of mouse cells. The researchers discovered that 216 genes were turned on at least three times more frequently in the stem cells than in other cells. "These 216 genes are likely to reveal core stem cell properties, or 'stemness,' that underlie self-renewal and the ability to generate differentiated progeny," they reported in the September 12, 2002, issue of *Science Express*.

When the scientists tried to find out where these 216 genes were located, they were surprised to learn that only 60 of them had been mapped to any chromosome. An astounding fraction of these latter genes—12 out of 60—sat on mouse chromosome 17, which also contains genes known to be

involved in embryo development and in sperm development. The 12, of course, will now be studied with special interest.

Another surprise was that the stem cells expressed unusually large numbers of "expressed sequence tags," which mark genes of unknown function. "For young scientists, this finding is especially exciting because it shows that ... no one has a clue to what the gene products do," says Melton. "It's easily a decade's worth of work just to define the functions of the genes that we have defined as characteristically active in these stem cells."

Although all stem cells expressed the 216 genes, they did so in varying proportions. "The three types of stem cells were not identical" in their activity, Melton points out. The activity of hematopoietic stem cells was more similar to that of other cells in the bone marrow than to the activity of any other samples, he says. By contrast, "embryonic stem cells and neural stem cells are much more similar to each

other than they are to their differentiated counterparts ... This fits with a 'default' model we proposed, which is that the default fate of embryonic stem cells is to become neurons."

The team's studies are likely to aid the search for new types of stem cells, Melton believes. "For example, nobody has yet been able to identify adult pancreatic stem cells—a central effort in our laboratory," he says. "But now we know that if we're going to isolate such cells, we should look for those that express many of these 'stemness' genes."

Melton is motivated by more than scientific curiosity. Hoping to find a cure for

his 10-year-old son, Sam, and millions of others with type 1 (juvenile) diabetes, he launched a major drive about a decade ago to turn human embryonic stem cells into the special kind of pancreatic cells, called beta cells, that supply the insulin diabetics lack. This effort has been slow going, he reports, but it did lead him to his stemness discoveries. —M.P.



MELTON

KATHLEEN DOORNER

scientists in Israel [led by Nissim Benvenisty of the Hebrew University in Jerusalem] who have experience in turning human ES cells into neurons,” Jessell says. The goal, he says, is “to find out whether you can efficiently convert human ES cells into human motor neurons.”

If so, “one could really start to evaluate whether this would be a sensible way of approaching ALS, spinal muscular atrophy or spinal cord injuries,” Jessell says. But he warns that there are big hurdles ahead. “The motor system relies heavily on the precision of connections and circuits. Simply having generated a motor neuron is not sufficient. I think the challenge will be to reconstruct appropriate circuits,” he declares. Partly for this reason, Jessell is now studying the next steps in the differentiation of motor neurons.

To help develop new therapies, Jessell has also started to collaborate with neurologists who are doing research on ALS as well as other spinal cord disorders and injuries. “There are many groups

interested in cell-based treatments of diseases like ALS,” he says, “but one of the difficulties in comparing results is that most people have been using different cells in different systems. If we can generate motor neurons under fairly standardized conditions, we can provide them to anyone who is interested, and it will be easier to compare their results.”

Jessell is working particularly closely with Robert Brown, director of the Neuromuscular Disorders Unit at Massachusetts General Hospital. “Bob Brown is a world expert on ALS,” Jessell says, “and he has mouse models of ALS. It will be interesting to test whether introducing motor neurons derived from mouse ES cells in these models actually does any good.”

The two research teams are also investigating the basic cause of ALS. A gene whose mutation is known to result in ALS in humans is the gene for an enzyme called superoxide dismutase 1, or SOD1. Brown uses a mouse model of ALS engineered with the same

Will Humans Generate Replacement Parts?

For the past six years, Mark T. Keating has been trying to figure out why humans and other mammals who lose a limb or an organ can't do what salamanders, worms and fish do in such cases: grow a new one. In mammals, cells that try to repair an injury just form a layer of useless scar tissue over it. But in newts, the cells that swarm over a fresh wound rapidly turn into a layer of stem cells that effect a complete repair.

Keating, an HHMI investigator at Children's Hospital in Boston, now believes this is the standard way that many animals regrow lost or damaged body parts. First, the wounds stimulate some mature cells to revert to their infancy as primitive stem cells, or “dedifferentiate”; then these cells are reprogrammed to form new tissue or organs. Certain genes are turned on to start both operations. According to Keating, “humans probably still have the genes that enable other animals to regenerate tissue, but these genes were silenced, turned off” during evolution. Therefore, they might be turned on again, given the right stimulus.

Keating and his colleagues identified their first such gene, *msx-1*, several years ago while doing research on human heart disease at the University of Utah. They discovered that *msx-1*

turns on in newts whenever these animals need a replacement part. Mice have a similar *msx-1* gene, and the scientists found a way to turn on this gene at will in mouse muscle cells.

Next, Keating, who had moved to Children's and Harvard Medical School by then, used a different stimulus—an extract of regenerating limb tissue collected from newts after their forelimbs had been amputated. This extract worked wonders on mouse muscle cells, making about 18 percent of them dedifferentiate and reenter the cell cycle—not very much less than the 25 percent of newt muscle cells that did the same. In their report on this work, Christopher J. McGann and Shannon J. Odelberg (both at the University of Utah) and Keating wrote that these studies “indicate that mammalian cells have retained

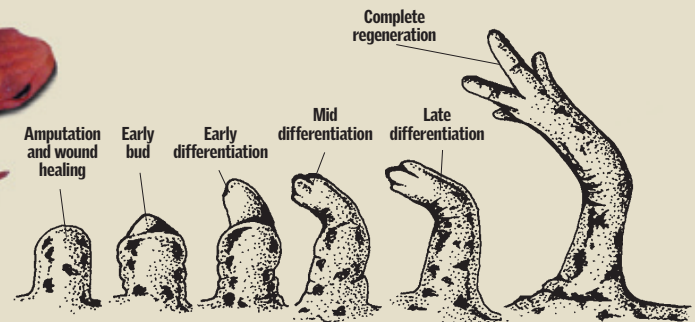
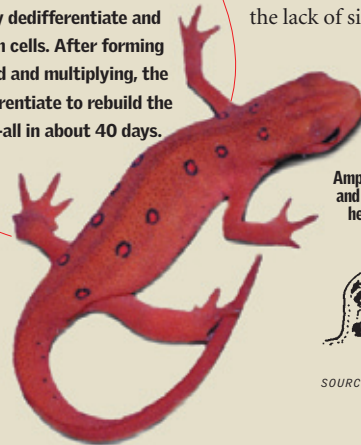
the intracellular signaling pathways required for dedifferentiation” and that the primary obstacle to regeneration in mammals may be the lack of signals to

start the process.

More recently, Keating and his associates compared how zebrafish and mammals react to heart injury. They found that zebrafish regenerate heart muscle with little scarring. However, other vertebrates respond by producing large connective-tissue scars. The researchers then proposed that “scarring and regeneration compete” and that the vigor of each process is critical. In normal zebrafish with heart injury, for instance, a fibrin clot formed, but cardiac muscle fibers “invariably penetrated the clot and constructed a bridge of new muscle around the wound.” By contrast, zebrafish with a mutation that impairs cell division produced fewer muscle cells and ended up with big scars. The researchers now believe that stimulating heart muscle cells to proliferate, in response to the proper genetic signals, “will reduce scar formation and facilitate cardiac regeneration in mammals as well.” Keating adds, however, that “it'll be a while before anything of this sort is tried in humans.”

—M.P.

FOLLOW THE NEWT
When a newt's limb is amputated, mature cells near the injury dedifferentiate and become stem cells. After forming a mound and multiplying, the cells redifferentiate to rebuild the limb—all in about 40 days.



SOURCE: DEPARTMENT OF ZOOLOGY, UNIVERSITY OF GUELPH, ONTARIO, CANADA

mutation, with resultant motor neuron degeneration. Yet “nobody knows why motor neurons are selectively vulnerable to death in ALS,” Jessell points out. He hopes to discover the answer by comparing motor neurons that are derived from normal ES cells to motor neurons derived from ES cells bearing the *SOD1* mutation. “Then we can ask what has changed, biochemically, that might be a predictor of the later degeneration of the motor neurons,” he says.

Scientists who work with stem cells are finding it difficult to choose among the many different research paths now opening up—and finding it particularly challenging to work with human ES cells. Although all mouse ES cell lines “behave in a rather uniform way, that may not be true in human ES cells,” Jessell says. Yet researchers may have a hard time finding out. Most of them rely on federal funds, which can be used to study only a limited number of human ES cell lines. And as Jessell points out, “human ES cell lines are so poorly characterized, compared to many of their mouse counterparts, that nobody actually knows how much they vary and whether the full range of developmental potentials is going to be offered in those lines that now have federal approval.”

WHAT ABOUT ADULT STEM CELLS? The researchers who have chosen to work with adult stem cells face other problems. In many tissues, adult stem cells are relatively unexplored and still full of surprises. They become most active when tissues wear out or are damaged, yet they are often hard to find. Besides the nervous system, researchers are looking at blood, skin, nails, hair, saliva and sperm cells—where the need for replacement is particularly obvious.

Allan C. Spradling, an HHMI investigator at the Carnegie Institution of Washington in Baltimore, Maryland, has focused on what he calls niches, special microenvironments in various organs such as the skin, gut and gonads. Each niche houses one or more adult stem cells and regulates their activity. His team has identified three types of regulatory cells that form such niches and keep the stem cells from differentiating prematurely.

The strongest evidence for such regulation in mammalian tissue probably comes from studies of spermatogenesis, Spradling says. Thousands of stem cell niches have been found lining the walls of the seminiferous tubules in which sperm develop. Spradling has focused on the niches that surround germline stem cells in fruit flies and on the signals they send to the stem cells. Such signals appear to have a powerful influence on the behavior of adjacent stem cells, he says. By comparison, the stem cells “may themselves be relatively unspecialized.” Therefore, the environment



**RIGHT CELL
FOR THE JOB**

Sean Morrison's team cultured these smooth muscle cells from neural crest stem cells of an adult rat.

They found the activity of the stem cells differed depending on their origin, pointing to the importance of matching the cell to the therapeutic goal.

of stem cells will have to be controlled with care if one hopes to use them in some form of therapy.

More data on the complexity of using adult stem cells comes from Sean J. Morrison, an HHMI investigator at the University of Michigan, who found that the properties of some adult stem cells were quite different from those of embryonic or fetal stem cells. Morrison managed to isolate “neural crest” stem cells from the gut tissue of adult rats, even though such stem cells (which give rise to various tissues, including the peripheral nervous system) were supposed to exist only in the embryo and fetus. Then he either cultured them or transplanted them into chick embryos to study their activity. It turned out that these adult stem cells could do some of the same things as embryonic and fetal stem cells but not others; for example, they could not differentiate into cells that make two important neurotransmitters, serotonin and noradrenaline.

Morrison also found that neural crest stem cells he had isolated from the gut of rat fetuses differed from those that came from the sciatic nerve. After transplanting cells from both sources into the developing nerves of chick embryos, Morrison discovered that stem cells from the gut produced mainly neurons, whereas those from the sciatic nerve made only glial cells, a type of supporting cell. This finding, he says, “suggests that it’s really important to match the origin of the stem cell to the therapeutic job that you’re trying to do.”

“There is a great debate in the field of regenerative medicine as to whether one should start with ES cells or with adult stem cells,” Jessell says. “Many groups are trying to get adult neural progenitors to differentiate into particular cell types. Our study shows very clearly that a mouse ES cell can become a motor neuron in a very predictable way, but so far, no one has shown that an adult neural progenitor cell can become a motor neuron.”

“I think the potential of adult progenitor cells is exciting,” Jessell adds, “but in my view, the evidence that they perform as well as their embryonic counterparts is not strong at the moment.”

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