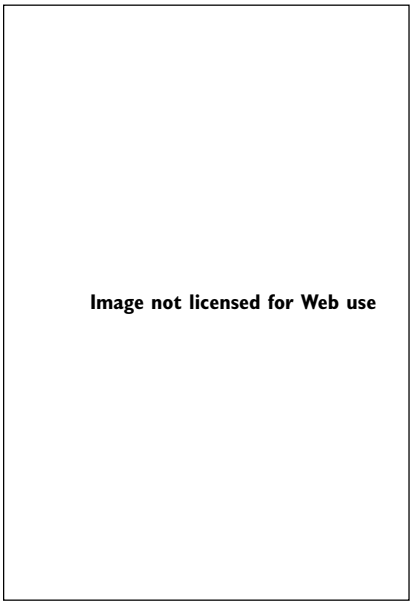
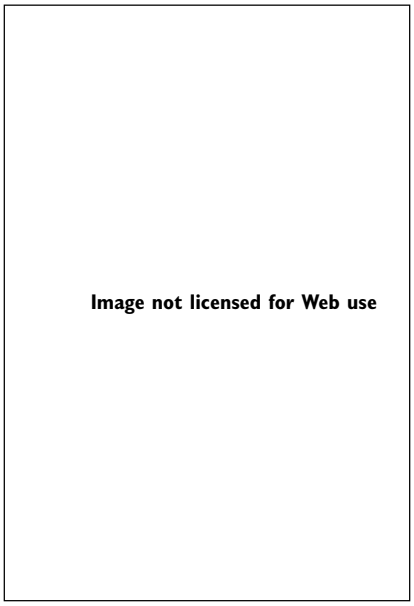
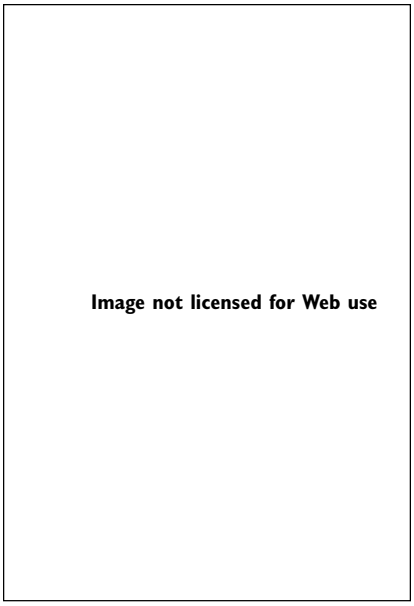
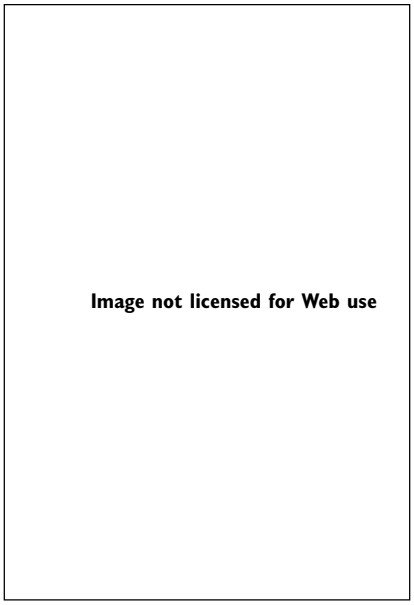
The kicker is figuring out how to steer the cells down one of those long, winding developmental pathways...to get what you want.

The sixth annual Caltech Biology Forum, held on February 24, was devoted to the burgeoning field of stem-cell research, which Science *magazine hailed last December as 1999's Breakthrough of the Year. This article is adapted from the remarks of three of the forum's speakers, who were joined by David Anderson, professor of biology and investigator, Howard Hughes Medical Institute, and by moderator Robert Lee Hotz, science writer for the* Los Angeles Times. *The event was cosponsored by Huntington Memorial Hospital, with which several Caltech faculty collaborate; and the San Gabriel Valley Newspaper Group, publishers of the* Pasadena Star-News.









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Barbara Wold

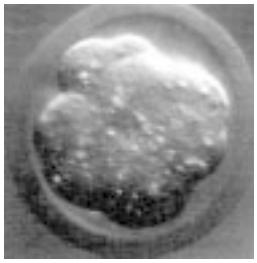
Professor of Biology Barbara Wold earned her PhD in biology from Caltech in 1978, and has been a faculty member since 1981. Her research focuses on the elaborate regulatory machinery that guides the development of muscle cells. She is also the director of the L. K. Whittier Gene Expression Center, established on campus in 1999, which draws scientists from several disciplines to the task of finding out what the roughly 100,000 genes in the human body do.

Left: This gallery of false-color scanning electron microscope pictures hints at the diversity of cell types obtainable from one totipotent stem cell. Clockwise, from upper left: a thicket of nerve cells and an astrocyte (green), which is also part of the nervous system; the epithelial cells that line the air sacs in your lungs; a smooth (involuntary) muscle cell; red blood cells, a T lymphocyte (green), which is a kind of white blood cell, and platelets (blue), which help the blood clot.

Stem Cells: The Science of Regeneration

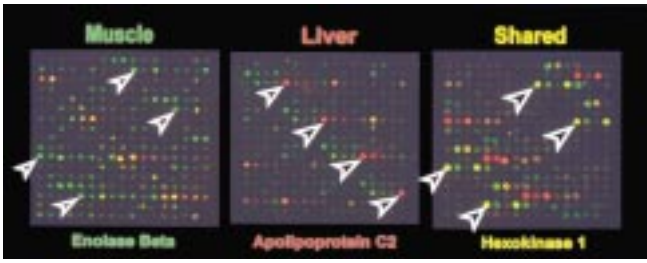
Above: A successful sperm sets off a rapid series of cell divisions in the fertilized egg, shown at two, eight, and approximately 40 cells.

It's hard to be a developmental biologist and not be fascinated by stem cells, which are "primitive" cells that give rise to other, more specialized, cell types. The story of stem cells is really two tales—one is the development of the embryo, and the other is regeneration in adults in response to injuries, degenerative diseases, and normal wear and tear. Many of our tissues—bone, the hematipoetic blood cell system, muscle—have to keep rebuilding themselves all the time just to keep us at steady state. Development begins when a sperm fertilizes an egg, and the cell begins to divide. You ultimately end up with many diverse cell types, even within one tissue. This raises an issue that will come up again and again—there is a big difference between replacing cells of a given type as a form of therapy, versus building a whole organ, like a kidney or a heart. The former we can begin to think about. The latter is way out there, and I think there's been some confusion in the popular press about this.

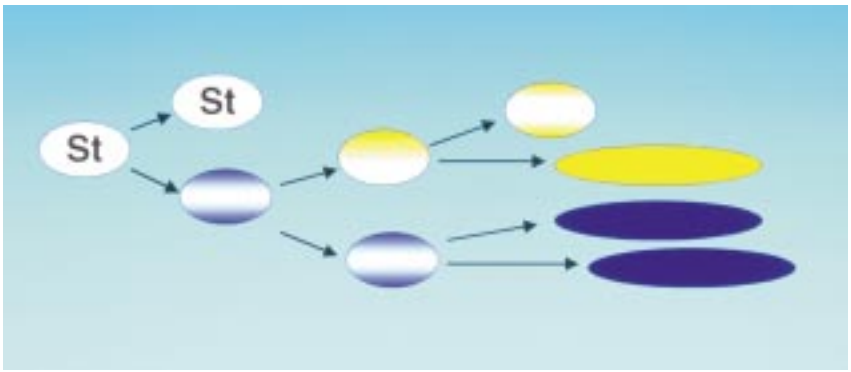


Cells come to be different through a series of stepwise changes in the pattern of genes that the cells express, or "turn on." The pattern can change in response to external signals, either from other cells—growth factors and hormones—or environmental cues. Or the changes may be programmed in the genes' DNA. So each time a stem cell divides (and sometimes even without dividing), internal and external signals cause it to choose from a progressively smaller number of paths. And at the very end of the trail are the so-called differentiated cells—red blood cells and white blood cells, the neurons in your retina, et cetera.

Differentiated cells have various shapes and functions because they express vastly different sets of genes. Each of your cells has a complete set of all the genes needed to make you—roughly 100,000 genes in all. Of those, maybe 5,000 are needed for the basic business of simply being a cell. In addition, each cell expresses maybe 2,000 to 4,000 genes that make that cell different from other cell types. We can now measure gene expression en mass, as shown below, in which a muscle cell is compared with a liver cell. The green dots on this microscopic chip represent genes expressed only in muscle, and the red dots, ones only expressed in the liver. Things common to both cell types come out yellow or yellow-orange. So we can look at vast numbers of genes



The arrows point to four copies each of enolase beta, a gene known to be specifc to muscle tissue; an apolipoprotein gene, similarly specific to liver; and a gene common to both cell types. Having this experiment correctly light up the genes we know gives us confidence that the genes we don't know are behaving the same way.



Stem cells remain like their parents or change into other cell types in response to internal and external cues. Here we have a totipotent stem cell ("St") leading to a pluripotent one (half blue) leading to a unipotent one (half yellow). The other blue pluripotent cell undergoes a gene-expression change without dividing, producing two differentiated cells in its next division cycle.

and figure out which ones are particular to each cell type and which ones are shared.

Now, stem cells aren't really any single kind of cell, but are cells that exhibit the quality of "stemness." "Stemness" is really a dual capacity—these cells can, at once, produce more progenitor cells just like themselves, and also produce other progeny that go on to assume the distinctive forms and functions of differentiated cells. This is usually done when a stem "mother cell" divides to produce two different daughter cells: One daughter retains the same properties as the mother cell, and the other goes on to acquire new properties.

There are three major classes of stem cells, based on what they have the potential to become. The earliest cells, from the fertilized egg through the first few division cycles, are totipotent—able to become any kind of cell under the right circumstances. You can grow them in a dish, and they will divide infinitely and retain this totipotent quality. Next come the pluripotent, or multipotent, cells, which can become more than one kind of cell but no longer contain the potential to become all cell types. And finally there are unipotent cell types, such as the muscle-cell progenitors that my lab works with. These still have the quality of regenerating, but have pretty well decided that they're going to become muscle. (Actually, our lab recently discovered that our muscle cells also have the potential to become fat cells, which is kind of scary when you think about it. I guess, deep down, we all knew this already.)

Since totipotent cells can become any kind of cell, it suggests a strategy for cell therapy—replacing cells in your body that have died or don't work properly as a consequence of some disease like muscular dystrophy, which is my field. Adding back stem cells has also been a very large part of the thinking about treating Parkinson's, and correcting certain diseases of the blood. The kicker is figuring out how to steer the cells down one of those long, winding developmental pathways through the many decisions to get what you want. Much depends on the cell type that you're trying to generate. And you may want to stop differentiation at a certain point and have the process finish once the cells are in the patient. But we have some pretty serious distance to go before we can do everything in reality that we can do conceptually.

I'd like to emphasize a distinction that I made earlier in passing—building an organ is a whole lot more complex than providing just one cell component, however important, of that organ. Growing blood or muscle progenitors is possible—it's done all the time with mice. Differentiating them is possible—we know enough about the right environments, in some cases, to nudge them in the right direction. But that's way different from growing a kidney or a heart. We're not even vaguely close to that. It would be very exciting, and I hope we'll eventually learn to do it, but it's pretty much in the science-fiction movieland realm right now.

One sometimes hears stem cells mentioned in conjunction with Dolly, the cloned sheep, and here's why: You could take the nucleus from one of your adult cells, as was done with an adult sheep, and fuse it with what's called an enucleated egg—one from which the nucleus, which contains the genetic material, has been removed—and implant it in a foster mother. Then you could make custom embryonic stem cells of your own personal genetic type for your own personal therapy. This would bypass the problem of tissue rejection, and the issue of where to find donors. Or, of course, you could essentially generate your own newborn identical twin, which is what Dolly

Why would you want to create an identical twin that's 20, 30, 40, 50 years younger than you?...I have yet to hear a persuasive argument for why cloning humans is a good thing to do, and it seems to me that most of the reasons for doing it are bizarre.

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is to the adult sheep from which the cell was taken. All the scientists I know consider doing this latter in humans to be totally unacceptable ethically.

Why would you want to create an identical twin that's 20, 30, 40, 50 years younger than you? The notion that this individual would be like you, in the sense of having had your experiences and sharing your thoughts, is clearly not the case. And think about the burden of expectation on, say, a clone of Einstein. What kind of life would that person have? There's also the issue of whether the clone would be healthy over a human life span, which is seriously in doubt. As the cells in our body age, they undergo changes to their DNA. For example, the telomeres—the ends of the chromosomes—get shorter, which appears to act as some sort of clock that may tell the cells when to die. Some of this is apparently reversible, or you wouldn't get all the way to Dolly. Nevertheless, it's not clear what the long-term prospects are. Furthermore, many of the cells in our adult bodies contain mutations that the fertilized egg didn't have. As we age, our DNA gets damaged by environmental factors, such as ultraviolet light, and errors can creep into the DNA when it replicates during cell division. Normally, this doesn't matter much—if a heart gene is mutated in a skin cell, who cares? It doesn't need that gene anyway. But when you make an entire human being from that cell, that person is at risk. Similarly, as we age, our cells individually accrue mutations of all sorts, including ones that lead in the direction of cancer without yet being frankly cancerous. Thus a cell can appear quite normal, and therefore be selected as a donor, but in fact vastly raise the likelihood that the cloned individual will develop cancer, and develop it at an early age. And if the clone has kids, the mutations will be passed on as part of their genetic patrimony, so we can really pollute the gene pool quite rapidly by introducing all sorts of genetic diseases. I have yet to hear a persuasive argument for why cloning humans is a good thing to do, and it seems to me that most of the reasons for doing it are bizarre. Most people are repulsed by the idea; I am one.

In conclusion, using embryonic stem cells for replacement therapy has some virtues and some liabilities. It solves the tissue-rejection problem. It guarantees donor availability. It offers the prospect of replacement of many different kinds of cells. On the other hand, there's the problem of providing the right signals—we know them for a few tissues but not for many. Every patient presents a different environment. And there's concern about unwanted genetic changes that might have occurred in your donor nucleus.

[Regeneration] is really very widespread among animals, and for reasons that are not understood, mammals have largely lost it.

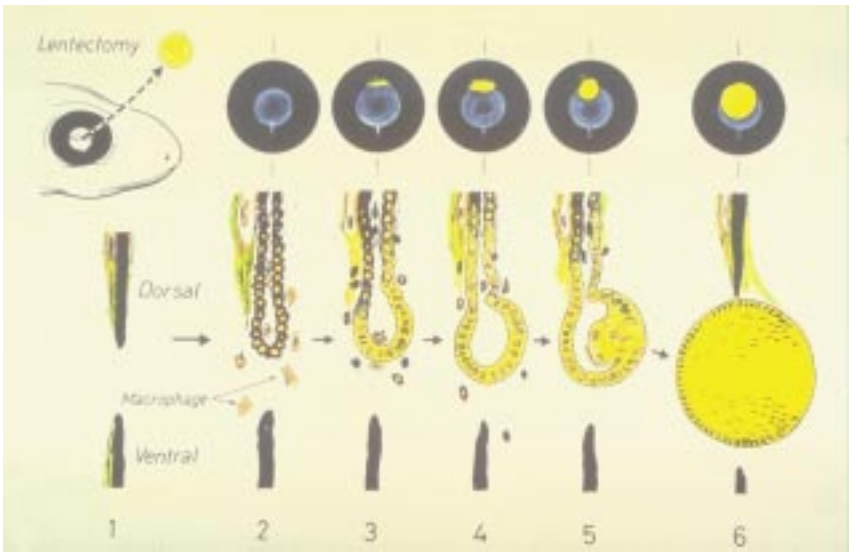
Jeremy Brockes

Jeremy Brockes, the MRC Research Professor at University College London, was a Caltech faculty member from 1978 to 1982. He has been given the Marcus Singer Award and a medal from the British Biological Council for his work on limb regeneration. He received his PhD from Edinburgh University.

I'm going to talk about what Barbara just called science fiction—using stem cells to regenerate whole limbs and tissues. As mammals, our abilities to do this are very circumscribed, with two notable exceptions—the regeneration of antlers in the male deer, and the regeneration of the liver. It's been suggested that an understanding of liver regeneration underlies the myth of Prometheus, who was cruelly punished by the gods by being chained to a rock while a bird devoured his liver by day, only to have it regenerate by night.

But even Prometheus's feat pales by comparison with the aquatic and terrestrial salamanders, who have the most remarkable regenerative ability among the animals that share our basic body plan. In the head alone, they can regenerate, with essentially perfect restoration of function, the upper and lower jaws and all of the ocular tissues—lens, retina, and iris. And the extremities—the limbs and tail—will regenerate as

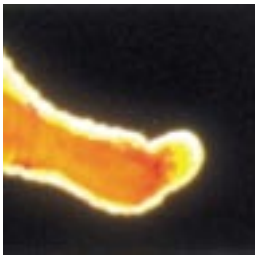
The arrows above point to just some of the body parts that a salamander can regrow with ease.

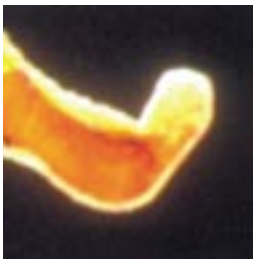


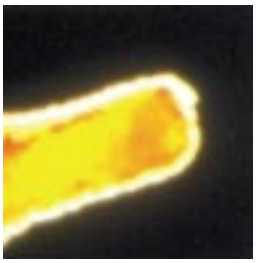
Left: This sequence of drawings shows what happens after a salamander's lens (yellow circle) is surgically removed. At bottom is a series of cross sections through the iris (black). Some of the black iris cells revert to stem cells, as shown by the color change, which then go on to form a new lens (the spherical structure), plus the muscle and connective tissue needed for it to function. This figure was kindly provided by Professor Goro Eguchi of Kumamoto University, Japan.

good as new, as will the internal organs, most notably the heart, which I'll mention later. Confronted with these feats, we tend to imbue these animals with an almost mystical ability. But this probably isn't the right way to look at regeneration. It's really very widespread among animals, and for reasons that are not understood, mammals have largely lost it. One argument for why we might have done so is that we traded it for the ability to heal wounds more rapidly.

Salamanders regenerate tissues by turning differentiated cells at the site of injury back into stem cells—a strategy we would like to learn. The drawing above shows how it operates in the case of the lens. After the lens is surgically removed, the pigmented cells of the iris change their identity, start to divide, and a new lens grows downward to replace the old one. It's a remarkably efficient reaction. But what's most striking, of course, is the complete restoration of an amputated limb, as shown in this sequence of photos.



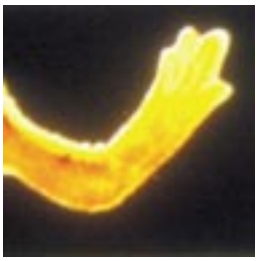


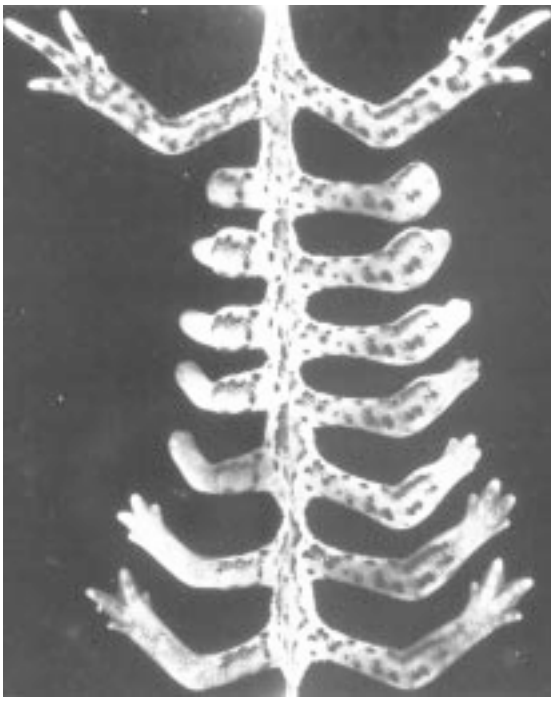


An amputated forelimb (above) grows back as good as new in 90 days (far right). Wish we could do that!

The animal generates a discrete population of stem cells—a growth zone, if you like—on the stump of the limb, and those stem cells reconstruct the missing appendage. This mound of stem cells, which we call a blastema, derives very important local cues for the function that it is going to perform. It's absolutely critical that the cells know to give rise just to the missing structures, whatever they are, and no more. So if the mound is at the shoulder, the stem cells somehow know to construct essentially an entire arm, whereas if they're closer to the wrist, they know to create only a hand. We don't as yet know what those cues are—what is it that cells derive from being at the wrist versus at the shoulder? Once the cells have been reprogrammed, the blastema has remarkable autonomy. We can cut it off and transplant it to, say, the tail fin, a region quite remote from the original limb, and it will still give rise to a perfectly normal limb. So we would obviously like to understand these processes, and manipulate them to our advantage.

The best we mammals can do along these lines is an experimental approach to bone repair based on stem-cell therapy. I have a colleague, Herve Petite, whose lab in Paris is deriving so-called mesenchymal stem cells from bone marrow and loading them into a scaffold that is positioned between the broken ends of a sheep's leg bone. (A newt can't repair such a gap in the bone.) The scaffold provides a mechanical guide of the appropriate shape, and also stimulates the mesenchymal stem cells to produce new bone that fills in the injury. Both functions are very important, and a lot of time and money goes into developing and evaluating new types of scaffolds for tissue engi-





Left: This is neither a coatrack nor some particularly appalling mutant cockroach, but two sets of composite pictures of a limb regrowing from the shoulder (left) and wrist (right). Below: The blastema is a tiny mound of cells, about 1.5 millimeters in diameter at the base. This one has been transplanted to the dorsal fin, where it will proceed to grow into a perfectly normal, albeit misplaced, limb. Photo kindly provided by David Slocum, Indiana University.

Above: A North American red spotted newt poses for the swimsuit edition of *Cell*.

neering. Interestingly, one of the most promising materials found so far is derived from coral. It's a natural matrix that is very good at stimulating stem cells to make bone.

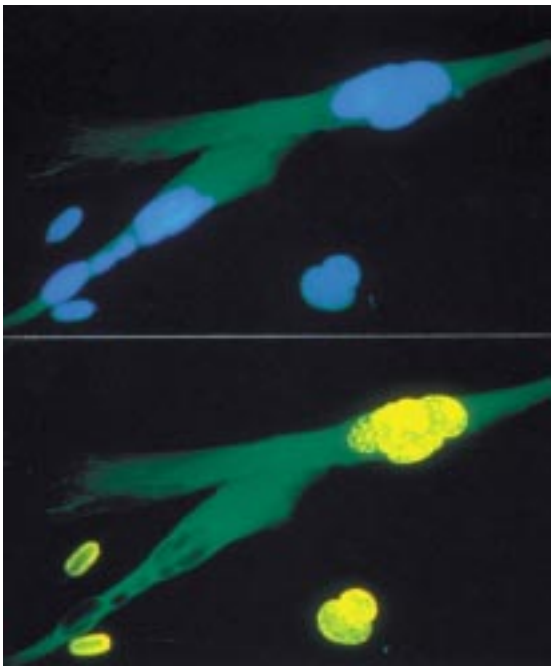
My lab studies the North American red spotted newt, which is a species of salamander, and I'm often asked, will we ever be able to regenerate like a newt? Unfortunately, there are so many layers of difficulty and uncertainty, so many things that we don't understand, that it's not possible to give a meaningful answer. When we're confronted with this sort of complex process, it helps to focus on particular parts of the mechanism that seem important.

I think a critical aspect of what the newt does is returning specialized cells to the cell-division cycle, which we mammals find very difficult to do. If you doubt in any way that this is important, let me reassure you with the example of the heart. Our ability to repair heart lesions is limited by the fact that our heart-muscle cells cannot respond to injury by dividing and generating more muscle. The newt, on the other hand, can respond to dramatic cardiac lesions by setting the cells around the lesion into division.

My lab studies this in the context of skeletal muscle, not heart muscle, but the principles are the same. Skeletal muscle arises from the fusion of cells with single nuclei (most cells have only one nucleus per cell) to give multinucleate muscle cells, which give rise to our muscle fibers. When this happens, our cells lose the ability to divide again. In fact, this is a general rule of differentiation—differentiated cells don't divide in response to the signals that caused their precursors to divide. But newt muscle cells can. At the bottom of the next page are two muscle cells from a newt.

The cell in the top photo has been stimulated to go back into the cycle of cell division, the first step of which is to duplicate the cell's DNA. That's indicated in the bottom photo by the fact that the nuclei are now yellow because they have taken in a fluorescent molecular precursor to DNA. Interestingly, we can fuse a newt muscle cell with a mouse muscle cell to obtain one cell that contains nuclei from both the mouse and the newt. And we find that if we stimulate the newt nuclei to enter the cell-division cycle, we stimulate the mouse ones as well. So whatever signal triggers the newt muscle to go back is also able, at least in this circumstance, to trigger mouse nuclei.

I'd like to end my talk on a personal note. I last spoke here 19 years ago, when I gave a public lecture on multiple sclerosis—one of the most mysterious and distressing of all the neurological disorders. (See "Nerve, Myelin, and Multiple Sclerosis," *E&S*, March 1982.) I was on the faculty here then, and I was working on a protein called glial growth factor that stimulated the growth of Schwann cells, which are the cells that form the insulation around your nerves and which are destroyed in multiple sclerosis. This factor has turned out to be very promising in stimulating the repair of the insulation, and in the last year, it's gone into clinical trials in human patients. These trials take three or four years, but it's been very rewarding to see that research come all the way to being tried for therapy. My hope is that the same will happen for our work on the newt and regeneration.



Alexander Capron

Alexander M. Capron is the Henry W. Bruce Professor of Law and the University Professor of Law and Medicine at the University of Southern California. He is a member of the National Bioethics Advisory Commission appointed by President Clinton, was chair of the U.S. Congress's Biomedical Ethics Advisory Commission, and before that was executive director of the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research under Presidents Carter and Reagan. He earned his LLB from Yale.

Ironically, using early embryos as a source of human ES cells turns out to raise more public-policy issues than using aborted fetuses.

The stem-cell controversy came to public attention in November 1998 when, in the space of a few days, there were three publications—two in scientific journals and one in the *New York Times*. The first, in *Science*, announced that James A. Thomson and his colleagues at the University of Wisconsin had generated lines of human embryonic stem (ES) cells from a frozen embryo donated by a couple who had received fertility treatment. At virtually the same time, John D. Gearhart's team at Johns Hopkins University announced in the *Proceedings of the National Academy of Sciences* that they had derived a special kind of stem cell, called an embryonic germ cell, from fetuses aborted about six to nine weeks after conception. (At this stage, the tiny fetus is still known scientifically as an embryo.) These two scientific papers were followed two days later by a *New York Times* story that scientists at Advanced Cell Technology (ACT), a Worcester, Massachusetts, biotech company, claimed to have created stem cells by fusing human cells with cow eggs from which the DNA had been removed. (However, it has been a year and a half now, and this work has never appeared in a peer-reviewed journal, so we really don't know what they did.) Because of the human origin of the cells involved in these three cases, President Clinton immediately asked the National Bioethics Advisory Commission (NBAC), of which I'm a member, to undertake a "thorough review of the issues associated with such human stem cell research, balancing all ethical and medical considerations." And so within just eight days we went from the first scientific announcement to the beginning of the commission's inquiry.

The Wisconsin, Johns Hopkins, and ACT groups each used a somewhat different method to produce their stem cells. The early stage embryo (or zygote) used by the Wisconsin scientists came from a fertility clinic that had used in vitro fertilization (IVF), the uniting of egg and sperm in the laboratory to create a "test tube baby." In the first

days after fertilization, the cells of the zygote are all totipotent, meaning that each one if separated could begin the process of creating an entirely new organism. (This is how identical twins are created.) The scientists at Wisconsin let the egg continue to develop for six to seven days after fertilization, until it took a form known as a blastocyst. A blastocyst resembles a balloon, on one part of whose inner wall is a clump of cells that are going to become the organism. (If the blastocyst were implanted in the uterus, as normally happens, the cells making up the balloon would become the placenta and the support structures for the developing organism.) Thomson's group cultured the cells from that inner cell mass to create a stable line of ES cells. Though no longer totipotent, these cells are still pluripotent: they cannot create a whole organism but they can give rise to all of the various specialized stem cells that are the origins of different tissues—bone, nerve, muscle, blood, and so forth. The ACT group also claims to have derived a human ES cell line from the blastocyst that arose when the fusion of a human cell with an enucleated cow egg cell caused the human cell to revert to pluripotency.

The other method, used by the scientists at Johns Hopkins, involved fetuses aborted at a stage in pregnancy when many women don't even know they are pregnant. Working through microscopes, the researchers went into these tiny entities to a structure called the gonadal ridge, which is made up of the cells that are migrating through the fetus to become the testes or the ovaries. These cells are called germ cells because they will beget eggs and sperm that can germinate into a new organism. These germ cells are still pluripotent, although they have gone a little farther down the developmental path than the blastocyst-derived cells.

Looking at these developments, NBAC saw a number of issues. The central issue was whether such work should be funded by the federal government or only be privately supported. Private sponsorship would take most of the public-policy issues off the table, but if private industry were the only source of stem cells, we could end up with trade secrets and exclusive licensing agreements limiting what scientists could do with the resulting cells.

You might well expect that the greatest controversy regarding public funding would arise over research using fetuses aborted at six to nine weeks, as opposed to embryos frozen less than a week after fertilization, and indeed debates about the ethics of research with fetuses have been going on for a long time. In the 1970s, a national research-ethics commission developed a set of rules and requirements for research involving cells and tissues excised from dead fetuses. The major requirement was that such fetal remains must be donated in accord with state law, which is pretty much the same across the country, since every state has adopted the Uniform Anatomical Gift Act. That act established a set of procedures for donating transplantable organs after death. It also gives the next of kin—in this case, the parents of the fetus—the authority to authorize the donation. Donations are supposed to be just that: gifts. That was further ensured by the National Organ Transplant Act, which specified that donors cannot be paid.

In the 1980s, physician-researchers began to try transplanting fetal neurological tissue to treat Parkinson's and other neurological diseases. It was proposed to the Reagan administration that the federal government fund this work, but abortion opponents worried that this use of fetal tissue would cause women to have abortions, or even to conceive a fetus for the purpose of providing fetal tissue to a relative with a neurological condition. So the Department of Health and Human Services (DHHS) was persuaded to impose a moratorium on such funding and to appoint an advisory panel of experts to study the situation. The panel recommended that the work be funded, but the department, by then under President Bush, rejected the recommendation and continued the moratorium. Nothing was done, however, to stop such research in the private sector.

When President Clinton came into office, one of the first things he did was lift the moratorium. To ensure that this new policy was implemented appropriately, Congress looked to the report by that DHHS advisory panel for relevant safeguards. Thus when Congress enacted the National Institutes of Health (NIH) Revitalization Act in 1993, federal funding of research involving the transplantation of tissue from aborted fetuses was permitted, but restrictions were placed on the manner in which these fetuses could be obtained. Not only were strict rules enacted about information disclosure and donor consent, but rules were established to remove any incentives for women conceiving fetuses for transplant purposes or deciding to abort just to provide tissue for transplantation. Specifically, researchers were forbidden to pay for fetal tissues or to promise that donated tissue would be transplanted into a particular person.

The upshot is that procedures and standards actually do exist for federal funding of research in which ES cells would be derived from aborted fetuses. Indeed, the Johns Hopkins team could legally have received NIH funding for their derivation of embryonic germ cells from aborted fetuses. (In fact, they did not rely on federal funds, but had private funding sources, including Geron, the Menlo Park, California, biotech company that supported the Wisconsin research.) So the only possible reason for amending the present statutes might be to make clear that the present rules, which apply to the transplantation of fetal tissue directly to patients, also encompass the use of such tissue as a source for ES cells for further research and eventual therapy.

PICTURE CREDITS:
16 — Bob Paz;
22 — USC

A blastocyst.

Ironically, using early embryos as a source of human ES cells turns out to raise more public-policy issues than using aborted fetuses, for a couple of reasons. First, taking the inner cell mass from a blastocyst ends the life of the embryo, whereas the gonadal-ridge tissue comes from aborted fetuses that are already dead. Second, the whole IVF field is in great ethical and legal disarray in the United States. It's worth noting that when IVF research began back in the 1970s, the British took quite a different approach. Their government appointed a body called the Warnock Committee—named for its chair, philosopher Dame Mary Warnock—which in 1984 recommended that IVF research be limited to the first 14 days of development, after which the embryos had to be destroyed. (Fourteen days is biologically significant, because it's before the embryo would implant itself in the uterine wall and before certain kinds of differentiation begin to occur.) Out of that came what was first a voluntary, and is now a statutory, licensing body in Great Britain. The Human Fertilisation and Embryology Authority (HFEA), which was set up in 1991, ensures that all licensed clinics that offer IVF or donor insemination, or that store eggs, sperm, or embryos, are inspected regularly and conform to high medical and professional standards. HFEA also licenses and monitors all human-embryo research, and serves as a forum for the debate that such research often stimulates. Because of its record-keeping requirements, the HFEA can actually keep track of how many embryos exist in all the labs and clinics in the United Kingdom.

The issue of supporting research on IVF actually received governmental attention in the United States before the Warnock Committee, but the results were less satisfactory. In May 1979, an Ethics Advisory Board set up by the Department of Health, Education, and Welfare recommended that the department should be able to fund IVF research under rules very similar to those later endorsed in the U.K. Yet in the U.S., the controversy that greeted this report was such that it has sat on the desk of any number of successive secretaries of what is now DHHS for going on 21 years. As a result we have no federal controls on human IVF research because the refusal to fund it at the federal level drove scientists who were working in this field to various sources of private money. Indeed, long before IVF was really ready for clinical application—or would have been used, had it followed the route of most research fields conducted with NIH support and leadership—a welter of private fertility clinics arose, where new procedures were tried out with patients' money.

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For a number of years, the limitation on federal support for research into IVF methods and other research involving the creation of embryos has been formalized through congressional riders to the appropriations bills for DHHS, which includes NIH. These riders provide that none of the funds in that statute may be used for research in which embryos are created, or for research in which they are "destroyed, discarded, or knowingly subjected to risks of injury or death greater than allowed for fetuses in utero." (Under federal research regulations, fetuses in utero cannot be exposed to any risk of substantial injury, except that necessary for their own benefit, which would obviously not apply to an embryo used in ES cell research.) In effect, federal funds cannot be used for the research whereby human embryonic stem cells would be obtained.

Plainly, government scientists—and many in universities, who are used to conducting federally supported research—want to conduct research using ES cells. The cells are viewed as enormously valuable—for basic research into cellular and organic development (including the biology of aging), for studies of drugs and other agents, and for therapeutic research, especially that which aims to control cellular development and to create replacement tissues (and perhaps even organs) genetically matched to the recipient. Does the rider absolutely prevent such research from receiving federal funds? NIH put this question to Harriet Rabb, general counsel of DHHS, who concluded that the restrictions do not apply to research *utilizing* human ES cells, for two reasons. First, "such cells are not a human embryo within the statutory definition"—being pluripotent, rather than totipotent, they cannot develop into a whole organism. Second, using an established ES cell line does not directly involve the destruction of an embryo. Therefore, NIH said it would fund research using, but not the research necessary to obtain, human ES cells. NIH established an ad hoc panel that prepared a set of rules for applying for federal funds for research with human ES cells that focused in large part on ensuring that the cells were obtained according to prescribed ethical standards. Many members of Congress vigorously protested this as a misinterpretation of their intent, while the American Association of Medical Colleges and other scientific groups strongly supported NIH's position.

Meanwhile, NBAC has concluded that it is intellectually indefensible to say that a statute intended to prevent federal funding of research in which embryos are destroyed would allow funding of research using the products of that process. It's obvious that when NIH funds go to a research group using stem cells, some of that money is going to go to the scientist in that group who's

creating the cells to be used. We also thought, for scientific reasons, that it would be far superior to confront the issue. If you artificially separate the process of deriving the cells and the process of using them, the scientists who use them cannot be directly involved in the method by which they are derived. Especially at this early stage of a field involving so many unknowns, how the cells are derived may turn out to have a great impact on how they behave and what can be done with them. So it would be natural—and scientifically preferable—for the scientists using ES cells to work closely with those deriving them.

NBAC's findings, released last September, concluded that two methods for creating human ES cells were acceptable. The first was to use aborted fetuses. This means induced abortions—spontaneously aborted fetuses are not a very good source, because a woman who spontaneously aborts at that developmental stage is usually unaware that she was even pregnant, much less that she has miscarried, making it nearly impossible to recruit such donors or to recover the fetuses. And as mentioned before, aborted fetuses are already an acceptable source of tissue for research, under federal regulations that impose strong consent requirements, separate the research process from the decision to abort, and prohibit any financial incentives that would lead the doctor or the woman to decide to have an abortion. The second acceptable source, we felt, was to use existing embryos from in vitro fertilization that have been frozen for some future pregnancy attempt. (In recommending that the prohibition be lifted on this specific category of embryo use, we did not address the general ban on embryo research.) The same strong consent requirements, separation from the research process, and ban on financial incentives that apply to fetuses should be erected here. Indeed, we argued that these embryos should only be made available after a couple has decided not to continue trying to get pregnant this way (or has decided that they have all the children they want to have) or after a particular embryo has been found to be unsuitable for implantation. This has since been proposed by Senator Arlen Specter, Republican of Pennsylvania, and Senator Tom Harkin, Democrat of Iowa, whose bill is now before Congress.

Nonetheless, we felt that it was not appropriate at this time for federal funds to be used in creating embryos specifically for research. First, there doesn't seem to be any need—there are hundreds of thousands of frozen embryos in storage; many, many more than would be needed to establish plenty of ES cell lines. Second, the possibilities for abuse are much greater, and the discomfort of many people with the prospect of starting a human life with the intention of ending it argues for restricting public funding. Of course, even under the regime we proposed, fertility clinics could intentionally create excess embryos. The best protection there, of course, would be to prohibit financial incentives and ban commerce in these embryos. Finally, we did not think it was appropriate, at this time, to make embryos from somatic-cell nuclear transfer—the process that gave rise to Dolly the sheep. Both the underlying cloning technology and the ability to get stem cells to differentiate into tissues and organs are still too rudimentary: this is a bridge we just don't need to cross right now.

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Some opponents of using ES cells have suggested that pluripotent cells should instead be derived by inducing differentiated adult cells to regress to a pluripotent state. (This would be somewhat similar to the research that Jeremy described.) It would avoid the need to create new embryos, and it could produce specialized tissues for autologous transplantation without resorting to somatic-cell nuclear transfer. Thus, this means of deriving pluripotent stem cells is very attractive, and work in this field deserves to be pursued and supported. But the research is still too preliminary, and the theoretical and practical barriers too great, to make it prudent to abandon research on deriving pluripotent stem cells from embryos and fetal tissues.

Three of our other recommendations bear particular emphasis. First, we urged ongoing oversight; any research using human ES cells should have to be approved by a review panel. Second, the protocols for deriving the stem cells should be reviewed by an Institutional Review Board, which is a research-ethics committee at the institution doing the work, to ensure compliance with requirements that would be established nationally. And finally, private sponsors of such research would be wise to adopt these same recommendations voluntarily, including submitting their protocols for deriving human ES cells to the national panel for review and certification. □