THE CRAFT OF ART | BY LEAH KAUFFMAN

THE EGG MAN

Delving into cloning, questioning popular fertility treatments, experimenting with embryonic stem cells, there's little Gerald Schatten's group does that isn't newsworthy. And the fact that his researchers work with our close cousins, monkeys, makes their efforts particularly relevant to human health. ABOVE: ANDi (backwards acronym for inverted DNA) as a baby. ANDi is the world's first transgenic primate, and techniques used to birth him from a petri dish may offer ways to create genetic disease models in primates. LEFT: A rhesus monkey (not ANDi) at the four-cell stage. A gene that makes a jellyfish glow was inserted into the DNA, illuminating one of the very early monkey cells.

While eating out, 3-year-old Gerald Schatten excused himself from his family's company. Peering over the edge of a neighboring table, perhaps pulling himself up on his tiptoes, little Gerald examined his fellow diner's lobster, then—for the benefit of any kindred amateur naturalists within earshot who shared his passion—pronounced its sex. But lobsters were just a warm-up: Throughout his school years, Schatten brought home scores of wildlife specimens found along New York's East River to identify with the help of scientific texts. He credits his forays along that waterway, set among one of the world's highest population densities, for giving him a precursor course in biology.

It's no wonder then that when Schatten went to the University of California, Berkeley, he majored in zoology. A doctorate in cell biology followed. He started with the study of sea urchin development and

Among those making things happen at the Pittsburgh Development Center: (from left) director Gerald Schatten, veterinarian Buddy Capuano, behaviorist Gerald Ruppenthal, and researchers Laura Hewitson and Christopher Navara.

progressed through the phyla: By the 1990s, Schatten was working with mice, cows, and pigs, investigating the molecular biology of the earliest stages of fertilization and development. In the mid 1990s, seeking an organism that provides an even more accurate reflection of human fertilization, Schatten began working with monkeys. Along the way his labs have been astonishingly productive in the field of reproduction, helping science answer befuddling cellular biology puzzles, like why mammals seem to inherit mitochondria only from their mothers.

Last year, he joined the faculty at the University of Pittsburgh School of Medicine. As director of the year-old Pittsburgh Development Center (PDC) at the Magee-Womens Research Institute and a professor and a vice chair in the Departments of Obstetrics, Gynecology, and Reproductive Sciences as well as Cell Biology and Physiology for the School of Medicine, Schatten continues the work he's long done on assisted reproduction technologies, the field known as ART. In recent years, Schatten's group has added cloning and transgenics to their repertoire, too. One of their findings may even end our national debate about the use of human embryonic stem cells for research and therapies. (Expect more on the embryonic front. As we went to press, we learned that the PDC had just received a shipment of human embryonic stem cells from the National Institutes of Health's tightly controlled registry.)

The treatment of infertility is considered elective by most American insurance companies. Since these treatments aren't covered by insurance, panels of doctors for insurance companies don't rule on which procedures are reasonable. The National Institutes of Health and other government agencies sponsor few clinical studies of ART and limited research involving human embryos. With a dearth of such investigations, patients may undergo infertility treatments without a clear picture of the risks to them, or to their child.

And there may indeed be risks. Evidence suggests that intracytoplasmic sperm injection (ICSI), a popular treatment for male infertility, may increase the likelihood of abnormal development, including chromosomal abnormalities, in the resulting children.

When a man produces too few sperm for normal conception, a couple might consider ICSI. The therapy involves using a miniaturized hydraulic apparatus to steady an egg while a sperm is drawn into a very fine needle, tail first. The needle then pierces the egg, depositing the sperm within. This often results in an embryo and then a baby, but Schatten's group is concerned that some of the molecular changes that take place during spontaneous conception are bypassed during ICSI, with uncertain results. For example, a sperm contains a protein collar that is shed as it enters the egg during spontaneous conception. Associate professor Laura Hewitson, who has collaborated with Schatten for the better part of a decade, helped show that during ICSI this collar is retained, cinching up the area where the sperm's X or Y chromosomes are contained, like a belt around a beach ball. This may prevent the sperm from integrating with the egg's genetic contribution. Another concern is that the mechanical manipulation of egg and sperm might cause damage. Hewitson discovered that in primate eggs, a structural landmark used by clinicians to guide the safe placement of the ICSI needle is not fixed as once thought. The technique of sperm injection, then, might cause chromatin damage. (Though it's a "big leap" to infer chromosomal damage from that stage, Hewitson cautions.)

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Since ICSI has been practiced in humans for a decade, there are plenty of ICSI children to study. Yet it may not be possible to detect subtle chromosomal abnormalities in kids so young, before their reproductive years. And it's notoriously difficult to determine the causes of any developmental delays in an uncontrolled setting. Are they genetic? Related to something in the environment? A result of the same thing that caused the father's infertility?

Because we share so much of our genetic makeup with monkeys, what Schatten and his colleagues at PDC learn from studying fertility treatments in them can likely tell us what happens when we undergo the same procedures, giving us a more accurate calculation of the risks. Likewise if their research determines that the risks are small, Schatten's group will be able to reassure clinicians and patients.

The continuation of the ART research depends upon healthy monkeys and their offspring, so the animals are never sacrificed for research purposes. And the PDC opts for minimally invasive procedures in its studies. In fact, while most embryo transfers into monkeys are done with minor surgery, Schatten's group, led by veterinarian Buddy Capuano, is working to perfect the use of a laparoscope instead. (In humans, the procedure

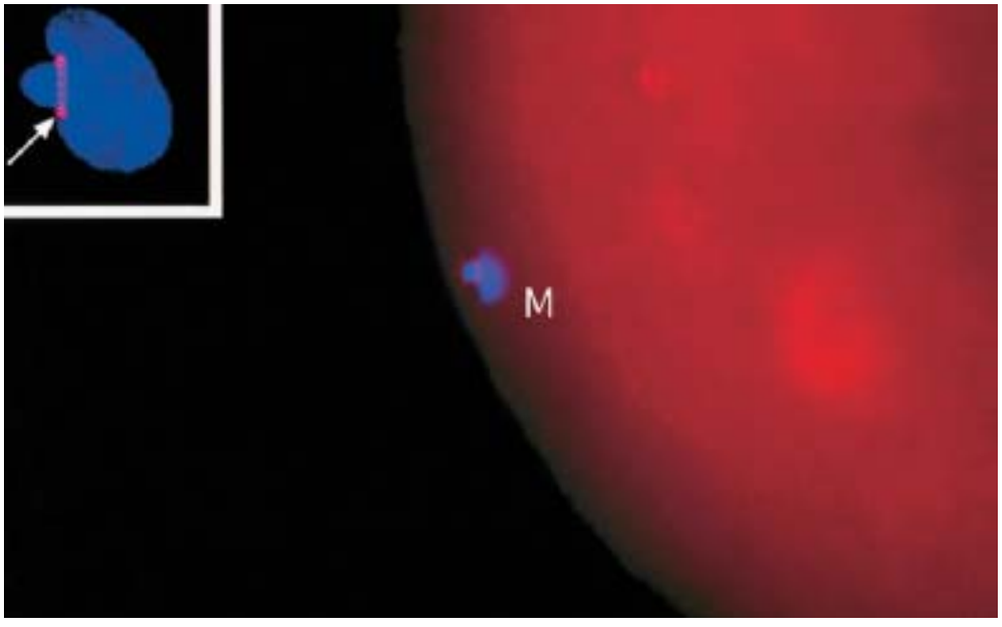
is typically done through the cervix, which is too difficult to navigate in rhesus monkeys.) The small incision required can be covered with a Band-Aid. Under the microscope, the PDC team studies what happens to egg and sperm just after conception. Then, using newer, more powerful imaging techniques, they track the development of a fetus in utero. Later, with the help of non-human primate behavior expert Gerald Ruppenthal, the monkey offspring are closely observed and assessed with a degree of scrutiny impossible in human subjects.

With more than 40 years' experience, Ruppenthal is known the world over for his work on monkey behavior, having edited an influential text on nursery-rearing monkeys (and he's a coauthor on another, to be published next year). By approximating mother rearing as closely as possible, by minimizing stress, and by providing crucial socialization and mental stimulation, Ruppenthal raises monkeys that know how to behave like monkeys.

The two Geralds met when Schatten was at the Oregon National Primate Research Center and Ruppenthal at the University of Washington. Ruppenthal's Seattle nursery, known for its extensive assessments of monkey infants, hosted some of Schatten's ART babies. The behaviorist calls the monkeys "kids." He enjoys watching them at play. Once they get to be about 6 months old, rhesus monkeys zing around like a cat with the evening crazies. They climb, leap, gallop, and wrestle. Harness their energy and you could power the lights of the Cathedral of Learning. In addition to play time with each other, they get intensive human handling in the form of daily physical and cognitive assessments, which not only provide data on development, but also give them intellectual stimulation in the form of puzzles to solve to find hidden treats and toys. More quickly than we can tell them apart, they recognize differences in us. Ruppenthal imitates their enthusiasm for a favorite caretaker by pounding his fist to his chest and leaning forward abruptly, a monkey ready to leap into the handler's arms. His widened eyes are the same cornflower blue as his broadcloth shirt.

Schatten is a considerable source of wattage himself. One day, setting up his laptop to display pictures of embryos and explain ART, he moves around his small, temporary office with hummingbird rapidity. (Next year, the PDC will get a new facility.) It's just as one Pitt administrator told Schatten it would be when recruiting him: The Pittsburgh research community is a scientific theme park, and Schatten is a kid with a ride-all-day, season-long pass. Already he has a roster of ongoing or hoped-for collaborations, with Chien Ho and Eric Ahrens over at CMU (MRI imaging), and Massimo Trucco, Steven DeKosky, Ronald Herberman, David Perlmutter, and Thomas Starzl at Pitt—to name a few. Tell him about a neat project going on up the hill, and he thinks of another potential collaboration. Inspiration is everywhere.

In 1998, inspiration came not from the East River but from the Atlantic waters off southern Massachusetts. Schatten had cofounded the six-week long program, Frontiers in Reproduction, at Woods Hole's Marine Biological Laboratory, meant to give physicians and researchers the most current, complete picture of the field. Staffed with instructors from all over the world, the program is an extremely intensive summer camp for young MDs and PhDs. Sometimes they don't leave the lab until the wee hours of the morning, they're so excited to be introduced to another technique or organism. Sometimes, they change career paths when they get home. Sometimes, even a faculty member is spurred to embark in a new direction. Exposed to an overview of molecular medicine that he would not have been otherwise, Schatten realized at Woods Hole that his group's work could help bridge the gap between experimental mouse studies and human cures. Monkeys have served as models for human infectious disease for a half century or more (the polio vaccine was tested in monkeys before it was used on humans), but they haven't yet been used as a



Laura Hewitson has found that a popular technique to help infertile men become fathers can cause unusual nuclear remodeling. The inset shows a protein forming a constricting ring around the male pronucleus, separating decondensed DNA from the condensed sperm head region. The red globe is the egg cell.

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model for a genetic disease. Could he create transgenic, clonal, and stem-cell derived monkey models that might be studied responsibly to accelerate cures for select and devastating human diseases?

To those accustomed to the fast pace of creating mouse generations, primate research is plate tectonics. (Rhesus monkeys are fertile only in the winter months and are pregnant for five-and-a-half months. Mice come to term within four weeks; they can overrun a facility before the invoice for the Purina Rodent Chow arrives.) So it was a relatively short duration in primate space-time before Schatten was able to present, in early 2000, Tetra, the first monkey, and first primate, made by embryo splitting, also known as artificial twinning—or "poor man's cloning" in Schatten's words. Tetra is one of quadruplet embryos produced by dividing an eight-cell embryo in four and injecting the cells into four empty egg shells (see *Science*, January 14, 2000). Tetra was the only offspring to result, but the technology that created her can help answer some pressing questions: Can stem cells cure diabetes and heal diseased hearts? How does the environment of the womb affect future development? And a load of other riddles.

In lab mice stem cells are spectacularly regenerative, healing gaping bone wounds and restoring muscle function after spinal injury. But those cells come from a few inbred mouse strains. No one knows if stem cells from a primate with a wider gene pool will have the same incredible plasticity. To answer this question, Schatten imagines that one monkey will be carried to term, while its genetically identical embryo provides stem cells that can be studied in its sibling (treating an injury or disease) without the complication of rejection. To help answer a nature versus nurture question, Ruppenthal imagines two identical embryos carried to term sequentially by different mothers, testing various hypotheses about the lifelong implications of conditions in utero.

A year after Tetra's birth announcement came ANDi's. ANDi (a backward acronym for "inserted DNA") is the world's first transgenic primate. Among his own rhesus monkey DNA, he hosts a jellyfish gene called GFP, an acronym for green fluorescent protein. GFP is a benign gene commonly used in labs because the protein it generates can be easily seen under fluorescent light. Though GFP makes jellyfish luminescent, ANDi doesn't glow, probably because his GFP gene isn't switched on and making proteins. In order to cause disease, a gene must produce lots of protein, all of the time. Since little ANDi isn't his own night-light, critics say Schatten is far from making a disease model monkey. (Another monkey in this project, although stillborn, did have fluorescent hair and nails.)

But this is an early effort, and Schatten considers ANDi a success. The techniques that Schatten's team used to construct and conceive ANDi in a petri dish will soon allow them to insert, for example, a gene that contributes to Alzheimer's into just the right place on a monkey's DNA so that the gene's function can be tracked.

Though ANDi proves that a foreign gene can be incorporated into the DNA of a primate, Schatten is far from encouraging scientists to abandon their lab mice and fruit flies for monkey models.

"These are our closest cousins," says Schatten. Biomedical research on primates deserves a great deal of oversight and thought, he says. In fact, he adds, many of the PDC's animal studies don't involve primates, but use sea urchins and rodents.

Transgenic primate models would be employed sparingly as well, as Schatten sees it: "We're working through advisory boards to ask—Are there certain diseases that we've learned so much about in mouse models, and the mouse model doesn't give us enough information to go to people?" He suggests breast and ovarian cancers as possible candidates for modeling. "Only old world primates and humans have monthly cycles. So we know a lot from the mouse world, but it's not yet enough. Or maybe cystic fibrosis. There are great models for cystic fibrosis in mice, but the diseases they get are different and they don't really get a lung disease."

Schatten sees his group producing transgenic monkeys, perhaps even identical, artificially twinned transgenic monkeys, when a cure for a given human disease is in sight, but still untested. One group would receive the trial vaccine or drug treatment while the other group, their twins, act as the experimental controls. The efficacy of the treatment could therefore be judged quickly. "And then we could get out of the monkey business," says Schatten. He predicts it will take at least two years of consideration, preparation, and lab work for the PDC to create the first transgenic monkey models of human disease.

Will the PDC's work bring us closer to a world in which humans are successfully cloned?

The idea is certainly unpalatable to Schatten, who points out that these technologies are designed to be used only as research tools to examine human disease or injury: "We do have ethicists who guide us at each step of the process. We're mindful of the ethical issues and do understand the dangers of extrapolating the science."

On the other side of the coin, maverick commentators, like Greg Stock, director of UCLA's medical technology and society program, think that human cloning by someone, somewhere, is unstoppable. While the use of federal funds for human cloning is banned, any privately funded effort to clone a human is legal. Stock imagines Schatten's work could make any future attempts less likely to result in the sort of physical and genetic abnormalities that have marked attempts to clone other mammals, many of which age—and die—prematurely.

Schatten couldn't disagree more with Stock: "Our work will show that human cloning is very unlikely to succeed and implement."

The PDC has discovered recently that cloning primates by nuclear transfer (the technique used to create the famous ewe, Dolly) poses even more difficulties than scientists imagined. They plan to publish their results in detail this fall.

And there is a windfall finding, reported, though overlooked, in the same *Science* article that introduced Tetra, which may resolve our national debate over the use of human embryonic stem cells.

The PDC team has shown that a few cells can be nipped off an eight-cell monkey embryo—the same technique used clinically for genetic diagnosis of cystic fibrosis and Huntington's disease—and used to culture stem cells. As with genetic diagnosis, the future development of the embryo is unaffected, meaning an embryo needn't be sacrificed for science or for life-saving therapies. Couples who undergo treatment for infertility might have the option of banking not only unused embryos, but—should they be needed one day to treat a family member's illness—precious embryonic stem cells as well. Every constituency in the debate over human embryo research can get its way: Patients can gain new therapies. Researchers can pursue promising experiments. Prolife activists can preserve viable embryos.

"Rather than debating who's going to win," Schatten advises, "be smarter and say, 'How can we all win?'" ■