Chimeras in the crosshairs

Just as human-animal chimeras are beginning to provide tantalizing insights into fundamental scientific questions about human disease and consciousness, conservative US legislators and President George W. Bush are launching a blistering moral attack. Christopher Thomas Scott investigates.

Near the end of George Bush's January 31, 2006, State of the Union Address came a short but pointed anti-science salvo. It was a familiar refrain, a religion-wrapped warning about reproductive cloning, embryo research and somatic cell nuclear transfer. But something new appeared amid the tangle of Bush's moral certitude and mistrust of science: the grim specter of human-animal hybrids, or chimeras.

Among those applauding in the House chambers was conservative Senator Sam Brownback (R-KS), author of the Human Chimera Prohibition Act of 2005 (**Box 1**). If passed, anyone who develops chimeras under the bill's sweeping reach would go to jail for up to ten years and pay a $1 million fine, presumably joining scientists, patients and caregivers put there by Brownback's Human Cloning Prohibition Act, which criminalizes the production of human embryonic stem (hES) cell lines and their use for future therapies. Those and four other stem cell bills, including Arlen Specter's (R-PA) legislation aiming to overturn Bush's 2001 restrictions, lie in front of the Senate. Stem cell backers are pressuring majority leader Bill Frist (R-TENN)—a recent convert to the benefits of hES cell research—to schedule a vote sometime this summer. Bush has threatened to veto any law that would override his policy.

Bush's provocative mention of "human-animal hybrids" was more than a rhetorical flourish, joining other loaded terms in the stem cell debate such as 'embryo' and 'cloning.' Like those words, biology's 'chimera' comes from years of common usage. The chimera of Greek fable was a fire-breathing monster with a lion's head, a goat's body and a serpent's tail, dispatched by the Pegasus-riding Bellerophon. By contrast, cellular chimeras are much tamer: organisms comprising genetically distinct populations of cells that hail from two or more zygotes. (Genetic mosaics, which are sometimes confused with chimeras, express different genotypes arising from a single zygote. Differential activation of the X chromosome, for example, produces the curious color of calico cats).

A chimeric menagerie

Chimeras have not so much arrived; they were already here. The world is home to a thriving menagerie of them. The joined fetal circulatory systems of a cow pregnant with fraternal twins will cause stem cells to seed the blood system of one twin with the other. In humans, placental blood exchange between the mother and fetus results in microchimerism, where progenitor blood cells from the child persist in the mother decades after the child is born[1]. Some estimate that up to 50% of mothers are naturally chimeric, and later-born children can carry cells of elder siblings that slip across the placental membrane during fetal development. In rare cases, a chimera can form after two embryos fuse. For example, in March, a US woman was denied welfare benefits after officials determined that her children's DNA did not match her own. Further tests revealed the mother was an amalgam of two genomes—her blood didn't match her children's, but cells from her internal organs did.

Despite the moral hand wringing in Washington, human-made chimeras have long been part of the scientific and medical scene. In the late 1980s, researchers at the University of California, Davis, transplanted the inner cell mass of goat blastocysts into sheep embryos, producing a female goat-sheep, or geep[2]. In the early 1990s, Nicole le Douarin at the College de France's Institut d'Embryologie in Paris put notochord precursor cells from quails into chicken embryos, making what might be called 'quickens' (marginally smarter, perhaps, than the barnyard fowl). The birds have since become the focus of dozens of experiments probing brain development, epilepsy and other disorders[3]. Other embryonic combinations have made mouse-hamster and mouse-rat chimeras.

In 2003, Chinese scientists made the first human-animal embryo chimera. Using cell fusion to transfer a human skin cell nucleus into enucleated rabbit eggs, researchers at the Shanghai Second Medical University made nearly 100 blastocysts. Upon the instructions of ethics advisors, researchers harvested the stem cells before 14 days. In January, Ian Wilmut announced his intention to use the Chinese technique to make lines to study human disease. Researchers in Douglas Melton's laboratory at Harvard University reportedly plan to deploy the method in advance of experiments that will

Republican legislators express their views on human therapeutic cloning during a House debate in 2003.

Box 1 Brownback proposes a blanket ban

If passed, Senator Brownback's Human Chimera Prohibition Act of 2005 would outlaw:

1. A human embryo into which a nonhuman cell or cells (or the component parts thereof) have been introduced to render its membership in the species *Homo sapiens* uncertain through germline or other changes. This provision would ban any transplantation into embryos aged zero to eight weeks.
2. A hybrid human/animal embryo produced by fertilizing a human egg with nonhuman sperm.
3. A hybrid human-animal embryo produced by fertilizing a nonhuman egg with human sperm. Routine *in vitro* fertilization procedures with hamster eggs to test sperm motility would be prohibited.
4. An embryo produced by introducing a nonhuman nucleus into a human egg.
5. An embryo produced by introducing a human nucleus into a nonhuman egg. This provision would ban the Chinese embryo fusion technique.
6. An embryo containing haploid sets of chromosomes from both a human and a nonhuman life form.
7. A nonhuman life form engineered such that human gametes develop within the body of a nonhuman life form.
8. A nonhuman life form engineered such that it contains a human brain or a brain derived wholly or predominantly from human neural tissues. This section bans all variety of human-neural mice.

transfer nuclei from patients with type 1 diabetes, Parkinson and Alzheimer diseases into human eggs.

The chimeric convention includes humans who benefit from modern medicine. Already walking among us are persons sporting pig or cow heart valves. Besides transplant patients with organs from unrelated donors, cancer patients who undergo hematopoietic stem cell transplants pump out two genetically distinct populations of blood, one of their own genotype and one from the donor. Surprisingly, drugs aren't needed to ward off rejection of the donor cells. And cells isolated from fetal pigs have been put safely into people with Parkinson and Huntington disease.

Now, using stem cells and our gathering knowledge of animal development (combined with fine microsurgical techniques), researchers may create new chimeric creatures. In general, the earlier in development one manipulates the embryo, the more profound the result. Put one donor cell into a recipient embryo of eight cells, and it will contribute in a major way to tissues and organs of the adult organism. Put a few cells into an animal with billions of cells, the effect is smaller—if there is a measurable effect at all. The medical implications of these feats are becoming clear: animal chimeras may become human organ donors and model organisms for science and drug discovery.

Reservoir pigs

Each year, thousands of patients die, waiting for an organ transplant. In 2001, for example, of the nearly 80,000 people in the US in need of a transplant, fewer than 24,000 got them; 6,000 died waiting. Four of ten bone marrow transplant patients will die waiting for a match. In the face of these statistics, how can donor programs keep pace?

One answer may be to grow organs and cells in animal hosts. The strategy involves manipulating the *in utero* fetus, before its immune system has a chance to learn the difference between 'self' and 'nonself.' Stem cells derived from embryonic lines or isolated from the body are transplanted to certain parts of the fetal anatomy. The full-term chimera has a tolerance to the foreign cells. Then, researchers assay the developing organs for human and animal cell markers.

Pigs may thus become both a model to study viral transmission and a vessel where human cells and organs grow. Jeffery Platt, director of the Mayo Clinic Transplantation Biology Program in Rochester, Minnesota, has carried out experiments in which human hematopoietic stem cells were injected into fetal pigs. Once the pigs were born, human cells were found in internal organs and throughout the blood system[4]. Over 60% of the nonpig cells were pig-human cell fusions—the first observation of its kind. Platt also found that whereas porcine endogenous retrovirus isn't normally infectious to people, the fused cells were capable of transmitting the virus to uninfected human cells. "We think viral and host cell fusion and genetic recombination turns on the virus," Platt says. "We noticed it only during one passage of cells—it didn't persist beyond that." This phenomenon may help explain how a retrovirus can jump from one species to another and shed light on the origin of diseases, such as AIDS and severe acute respiratory syndrome.

The Mayo group hopes that pigs can become human cell 'incubators.' The chimeric pigs produce a diverse population of human T cells, and Platt plans to use microsurgical techniques to precisely place human adult stem cells into budding organs, such as kidneys or livers in an animal fetus. Provided fusions won't diminish the number of usable cells, human cells purified from the chimeric organs could be used as cell therapies.

Although xenografts carry some risk of zoonosis (the transmission of an animal virus to uninfected human cells), Platt, a surgeon, reminds us that zoonotic infections can be controlled and the objective "is to generate organs that are lifesaving." Esmail Zanjani, a researcher at the University of Nevada at Reno, agrees with Platt's assessment. Using the fetal transplant method, he injects embryonically derived human hematopoietic stem cells into sheep. The technique produces an animal with organs, including the liver, heart and pancreas, that are 15% human. The cells persist seven years later, and the human cells from one sheep can be transplanted into another and survive[5].

"We want to use the animal as a concentrator," explains Zanjani. "First, we need to figure a way to purify progenitor cells of a patient's liver or heart, and inject them *in utero*. Then, we hope that they travel in the animal to the site of organogenesis." In this hopeful scenario, a part-human (but tissue-matched) and part-xenogenic organ is transplanted. The patient rejects the part that is animal while retaining the autologous part. Zanjani adds that sheep-human blood chimeras make excellent model organisms because of the similarities between the blood forming systems of the two species.

The human side of disease models

Laboratory chimeras may help unravel the mysteries of human disease without risky and unethical human experimentation. In 1988, researchers at the Palo Alto, California, biotech company SyStemix (later acquired by Novartis of Basel) developed the SCID-hu mouse, a severe combined immune deficient (SCID) mouse into which functional human lymphoid tissues (such as thymus and

lymph nodes) had been engrafted. This type of approach has been used widely to create experimental SCID mouse models to study not only the human immune system but also other organs, such as skin, lung and colon. A recent experiment produced a chromosomal chimera: a research team in the UK reported last September they had created a line of mice with human genes for Down syndrome that exhibits behaviors, organ size and neuronal numbers that mimic the human disease. They extracted chromosomes from human fibroblast cells and transferred them into mouse embryonic cells. The transgenic cells were then put into early mouse embryos, where foster mothers carried them to term[6].

Now scientists are putting human neural stem cells into animal brains to probe dementias and other neural disorders. The Burnham Institute's Evan Snyder first put human fetal neural stem cells into mice in 1998 (ref. 7). The cells formed self-renewing clusters called neurospheres—a stem cell signature—and changed into mature neurons, astrocytes and glial cells. Later, he transplanted neural cells into murine models of cerebral palsy and stroke. In both cases, the stem cells repaired the damaged areas.

"Following the path of neural cells in little mouse brains is really difficult," says a bemused Snyder. Migration or 'homing' is a big deal to stem cell biologists, because a stem cell that homes effectively can be transplanted distally without harming the organ. Snyder puts stem cells committed to the neural lineage into the brains of monkeys with Parkinson-like syndrome and motor disease, experiments that raise eyebrows among some bioethicists. The cells travel the distance and not only establish residence in the area of interest, but produce cell types that restore lost function.

This year, the Salk Institute's Fred Gage announced that he had made mice with brains containing 1% human cells[8]. He injected fluorescently labeled hES cells from a local biotech company, CyThera, into the lateral ventricles of 14-day old fetal mice, and followed their fate. Not only did the cells survive, they changed into neurons, proving that hES cells placed in the proper microenvironment can fully differentiate. Gage found the human cells made synaptic connections to mouse neighbors and fired properly. The big news, however, was the absence of teratomas, the unorganized, nonmalignant tumors that result when hES cells are placed in the body cavities of mice. A worry about using hES cells or mixes of cells derived from hES cells is tumor formation in sick patients—a cure worse than the disease.

The good news about the absence of tumors is tempered by the fact that the cells rushed headlong into terminally differentiated types. "We didn't see any proliferative cells—a slowly dividing population of neural stem cells we could use as a model system," remarks Gage. "That is disappointing from a scientific point of view." Looking ahead, Gage and his group want to develop lines of hES cells with human neurological disease, and thereby transfer the pathological effect to a strain of chimeric mice. A whole-animal model would have distinct advantages over disease-based lines of hES cells for testing drugs or studying the progression of brain illness.

Paws for thought

With an animal model in mind, Stanford's Irving Weissman proposed thought experiments in 2003 to make mice with brains that were partly or wholly human. This caused a stir among bioethicists and scientists. Weissman had shown earlier that human neurons could engraft into the sub-lateral ventricles of SCID-hu mice and move into other areas of the brain. But he couldn't tell if the cells functioned properly.

Weissman suggested two experiments to answer this question. One would put human neural stem cells into a strain of SCID-hu mice where the neurons in the cerebellum degenerate several weeks after birth, resulting in severe motor deficits. (The human analog of this disorder is called Fredrick ataxia.) If the mice improved after the transplant, it would show the engrafted cells were functional. The second experiment planned to use a mouse with a bigger problem. Harvard's Fred Alt had made a line of mice where all of the neurons die a week before birth, killing the fetus. Weissman reasoned that transplanting neural stem cells *in utero* could yield a surviving mouse with a brain made of human cells.

> "Tonight I ask you to pass legislation to prohibit the most egregious abuses of medical research: human cloning in all its forms [including] creating human-animal hybrids."
> G.W. Bush.

Before proceeding, Weissman asked a panel of Stanford bioethicists and scientists to evaluate his protocols, especially the second. "I thought, would the brain's organization be mouse or human?" he recalls. "Are the differences at the single cell level, dependent on the size of the brain or related to the brain's architecture?" His ruminations begged an ethical question: would mice made of human brain cells have human consciousness?

Snyder, whose own experiments with Parkinson monkeys show human stem cells can improve symptoms, doesn't think so. "Nervous system function isn't tied to one cell, it's the entire milieu that's responsible. To restore function you need to replace other things, too." Snyder—a pediatrician who studied philosophy and linguistics—explains that during early development, hES cells are instructed to build organs and systems, not just replace cells. He adds, "Human neural functioning is more than the sum of its cells. Being human can't be reduced to component parts."

Gage's recent experiments on hES cells in the brains of mice resolved some questions about neural function. The hES cells appeared to be fully integrated, with axons connecting to mouse cells. He also recorded action potentials between the cells, but doesn't know if they approximate human or mouse signals. If the human-mouse neural connections cause some to worry, Gage says the cells look more mouse than human. "We see the human cells approximate the shape and size of mouse neurons. This tells us the human cells are being governed by the microenvironment of the mouse's brain."

Could human neural stem cell transplants transform animals into creatures with human consciousness? Although it can't be ruled out, it seems unlikely. Mouse brains are much smaller and organized differently than their human counterparts. Stanford's Hank Greely, who was in charge of reviewing the Weissman experiments, uses bricks and blueprints as examples. "You can build a gas station and a cathedral using the same bricks; what makes them different is the architecture."

Raw numbers of neurons are important, too. Robert Sapolsky, a neuroscientist also at Stanford, explained the differences between chimpanzees and humans in a recent essay. "Our braininess as a species arises from having humongous numbers of just a few types of off-the-rack neurons and from the exponentially greater number of interactions between them," he wrote. "The difference is sheer quantity"[9]. Nonetheless, Greely's panel recommended that although the experiments could ethically proceed, researchers should carefully observe the mice for any unusual brain structure or behavior. At this writing, Weissman has not begun his experiments.

Shades of gray

Despite Bush and Brownback's assertions, the bioethical dimensions of chimeras aren't black and white, but range along a continuum. Ethicists agree that grafting some human neural stem cells into fully developed nonhuman primates is not likely to cause ethical worry. But there are several areas that require vigilance on the basis of our understanding of embryonic life and our close relations to nonhuman primates. First, before transplanting primordial cells—human into animal or animal into human—red flags are raised if the cell is put into an early embryo, when the developmental program is still in flux. Some might feel queasy meeting a sheep with human features. Related to this is a more delicate issue—the gonads. There is a chance, for example, that hES cells could enter the germ line of a mouse and produce human eggs or sperm. For that reason, animals in which hES cells are introduced should not be allowed to breed. Finally, go slowly when putting human neural stem cells into nonhuman primate brains, lest the procedure instill some unknown level of human consciousness. On the last point, scientists and those who regulate chimera research should pay attention to the number of cells transplanted, the site of engraftment and the brain size and species of the recipient. For instance, millions of human cells transplanted into a gorilla would raise greater concern than thousands of cells put into a marmoset.

The 2005 US National Academy of Sciences *Guidelines for Human Embryonic Stem Cell Research* recommends that a committee of ethicists, embryologists and other experts first approve protocols that plan to put hES cells into nonhuman animals at any stage of development. The guidelines state outright that no experiment be permitted when hES cells are introduced into nonhuman primate blastocysts[10]. Alta Charo, a University of Wisconsin bioethicist who was one of the authors of the National Academy report, puts the problem in clear terms: "We should not put a human embryonic stem cell into a primate blastocyst because it might humanize animals in ways that are unnerving. We just don't know how to manage this yet."

Other philosophers react negatively to the creation of human-nonhuman chimeras. This gut response—though it is hard to articulate—is advanced as reason enough to consider the practice wrong. Presidential ethics advisor Leon Kass believes that the creation of chimeras is a moral taboo. Others claim that such creatures are 'unnatural' and go against our norms of what defines a species, declaring that chimeras introduce moral confusion into our ways of thinking about humans and our relation to animals. Last year, a panel of ethicists convened at Johns Hopkins rejected these views, citing examples of xenografts and pig-to-human cell therapies. The line between the natural and the unnatural is impossible to measure, they argue, as most of modern medicine cannot be found in nature[11].

In the end, society's wish to make animals that serve the interests of science and medicine will be weighed against our best guesses about the kind of creature we create using stem cell transplants. The discussion will likely move past mice with human neural cells and pigs with human blood to other questions at the boundaries of science and medicine. Will knowledge gained from brain chimeras lead to treatment of human dementias or illuminate ways of altering human consciousness? Will stem cell therapies create an accidental genius? How would we feel if a great ape could understand the beauty of a Monet painting or weep during a Mozart concerto?

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1. Bianchi, D.W. *et al. Proc. Nat. Acad. Sci. USA* **93**, 705–8 (1996).
2. Roth, T.L. *et al. Biol. Reprod.* **41**, 675–682 (1989).
3. Le Douarin, N.M. *Trends Neurosci.* **16**, 64–72 (1993).
4. Ogle, B.M, *et al. FASEB J.* **18**, 548–550 (2004).
5. Almeida-Porada, G., Porada, C. & Zanjani, E.D. *Int. J. Hematol.* **79**, 1–6 (2004).
6. O'Doherty, A. *et al. Science* **309**, 2033–2037 (2005).
7. Flax, J.D. *et al. Nat. Biotechnol.* **16**, 1033–1039 (1998).
8. Muotri, A.R. *et al. Proc. Nat. Acad. Sci. USA* **102**, 18644–18648 (2005).
9. Sapolsky, R. *Discover* **27**, 42–46 (2006).
10. National Academy of Sciences Guidelines for Human Embryonic Stem Cell Research (The National Academies Press, Washington, DC, 2005).
11. Greene, M. *et al. Science* **309**, 385–386 (2005).