

# Hatching the Golden Egg: A New Way to Make Drugs

After 2 decades of work, researchers have succeeded in creating gene-altered chickens that can lay eggs containing human proteins

Chicken farms are among the most pungent places on Earth, but if a few hardy entrepreneurs have their way, it may soon be the sweet smell of success that wafts from buildings housing thousands of transgenic hens. As many as a half-dozen small companies are hoping to turn the common chicken into a pharmaceutical bioreactor, one that can meet the growing demand for protein-based human therapeutics.

The idea is deceptively simple: Insert human genes into chickens and get them to make human proteins in egg whites. "Transgenic chickens should be near-perfect bioreactors for making large amounts of pure recombinant proteins," says Ann Gibbins, an avian biologist at the University of Guelph in Ontario, who did the pioneering studies in the field and trained many of those scientists now achieving success. But, as she found out the hard way, putting the scheme into practice has been tough. Now, after 2 decades of struggles, researchers are reporting numerous successes in making transgenic chickens. The next-generation bioreactor should follow close behind.

As far as the drug industry is concerned, such transgenic chickens will most certainly be laying golden eggs. It's just a matter of time, drug companies say, before they will be riding a wave of new health products based on manufactured human proteins. Already, they have concocted more than 300 human antibodies in the lab and begun testing them against a variety of human ailments—from cancer to viral infections to immune system disorders. But the companies need a better way to make these products. Current methods can be inefficient: The biotech firm Amgen, for example, has been having trouble meeting demand for its arthritis medication Enbrel, which contains a human protein made by Chinese hamster ovary cells.

The math of making drugs in chicken eggs is appealing. Each commercial hen lays

about 250-plus eggs a year, at a nickel apiece. Each egg contains nearly 4 grams of egg white, comprising a mere eight proteins. Get a transgenic chicken to add 100 milligrams of a recombinant protein to that mix, Gibbins says, and the final cost for purified protein should be about \$10 per gram, 100-fold less than the cost of current systems using cultured mammalian cells. Compared with other proposed animal production systems—cows or goats, for example—chicken flocks



**Rich brood.** When mature, transgenic chickens are predicted to be more efficient producers of human proteins than current pharmaceutical bioreactors.

are easy to ramp up in months. There are other benefits as well: Commercial egg farms are already secure enough that a transgenic chicken, for instance, is unlikely to escape into the wild and breed. And there's a bureaucratic comfort factor: The U.S. Department of Agriculture and the Food and Drug Administration are familiar with eggs as bioreactors because many vaccines, including those against influenza, are already produced in chicken eggs, although not transgenic ones.

Human biology also suggests why egg-reactors should work better than alternatives such as bacteria or nonhuman mammalian cells. "Chickens add sugars to finished proteins in much the same way that humans do, something that can't be said for most other protein production systems, current or envisioned," explains virologist Bill MacArthur, president of GeneWorks, a chicken research firm in Ann Arbor, Michigan. Proteins fin-

ished with the human glycosylation pattern are more active biologically.

But for all the potential benefits, "creating a transgenic chicken has proven to be a far greater challenge than we ever expected," says Gibbins. Although reports of success have appeared in the literature every couple of years, none has panned out. Either the introduced genes failed to carry over into later generations or the chickens failed to produce the desired protein in their eggs. "It left us all thinking that it was going to take some kind of magic to ever make a transgenic chicken egg."

The magicians have arrived. At least three research teams using different methods have now shown that they can make transgenic chickens in proof-of-principle experiments. "Nobody has a transgenic chicken ready to produce a pharmaceutical today, but the field

has now gotten to a place where this will happen," says developmental biologist Jim Petite, whose group at North Carolina State University, Raleigh, developed a transgenic chicken that produces the bacterial enzyme  $\beta$ -galactosidase in its eggs.

## Needle in a haystack

Much of the trouble in creating a transgenic bird arises from some unusual features of avian reproductive biology: the sheer size of the egg and the difficulty of harvesting one before it has begun growing into a chick. The most common way to make a transgenic animal is to harvest a newly fertilized egg and inject foreign DNA directly

into this single cell's nucleus using a microscope and an ultrathin syringe needle. Eggs are easy to harvest when laid, but by that stage the zygote has already grown into a 60,000-cell mass. Harvesting the zygote from the chicken's oviduct, difficult and expensive in itself, does yield a single cell, but one almost impossible to find within the viscous, yellow yolk.

Helen Sang, an avian molecular biologist at the Roslin Institute in Midlothian, U.K., is one of the few researchers in the world to have figured out how to harvest a newly fertilized zygote and inject it with DNA or manipulate the 60,000-cell zygote of a freshly laid chicken egg and still get it to hatch into a transgenic chicken. Working with researchers from Viragen in Plantation, Florida, Sang has successfully created transgenic chickens that express a green fluorescent protein and are able to pass the introduced genes on to subsequent generations.

In this proof-of-principle study, Sang's team used a lentivirus-based gene delivery system engineered at Oxford BioMedica in London to transfer the gene for green fluorescent protein or for  $\beta$ -galactosidase into the DNA of chick embryos from freshly laid eggs. The injected embryos were then transferred to a host shell whose top had been carefully removed. Plastic wrap sealed the breach in the eggshell, allowing about 30% of the reconstructed eggs to develop and hatch normally. Of the eggs that hatched, 10 were transgenic roosters, evidence that the introduced gene had incorporated itself into the chicken's DNA. Upon mating, each rooster successfully produced up to 29% transgenic offspring expressing the green fluorescent protein or  $\beta$ -galactosidase, which Sang characterizes as "remarkably successful ... far better than anything we've seen before." Using Oxford BioMedica's lentivirus system, she is now developing a transgenic chicken designed to express a "clinically relevant hu-

man monoclonal antibody." By linking the gene for this antibody with the promoter for one of the egg-white proteins, Sang hopes to have it expressed in commercial quantities within the egg white.

Rather than try to manipulate a chicken egg, researchers at BioAgri in City of Industry, California, are working with rooster sperm. The key to this strategy is a monoclonal antibody, developed by BioAgri scientists, that binds specifically to the surface of sperm and allows DNA linked to the antibody to enter the sperm cell and incorporate itself into the sperm's genome.

Using this sperm gene-transfer technique, developmental biologist Jin Qian and his colleagues have created two different transgenic chickens that produce human interferon  $\alpha$  and interferon  $\beta$ . The BioAgri researchers have shown that the interferon genes are stably transmitted across two generations of offspring so far. In addition, their subsequent study using green fluorescent

protein linked to the lysozyme gene promoter demonstrated successful gene expression in egg white. The chicks from that experiment are nearing maturity and will be bred to see if this gene is passed to the offspring.

Based on their successes so far, both Viragen, which supports Sang's work, and BioAgri are now negotiating with pharmaceutical companies to develop transgenic chickens that will make human proteins for clinical development. Petite, who is not associated with either firm, wouldn't be surprised if these deals were inked soon, a sign that this field is finally realizing its promise. "In the late '80s, lots of pharmaceutical and biotech companies had transgenic chicken programs because everyone saw the potential for lowering production costs, but they all got off the field when their programs went nowhere," says Petite. "Fortunately, some of us were stubborn, and now the payoff is here. It's time to be optimistic." —**JOE ALPER**  
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## Cosmology

# With Its Ingredients MAPped, Universe's Recipe Beckons

Now that the Microwave Anisotropy Probe has nailed down what everything is made of, researchers are scrambling to figure out how it all came together

**DAVIS, CALIFORNIA**—A month after the first pictures from the Wilkinson Microwave Anisotropy Probe (the satellite formerly known as MAP) pinned down the fundamental constants that describe the cosmos, prominent stargazers and theorists gathered here to gear up for the next phase in cosmology.\* All agreed that their field was far from played out.

For years, physicists using balloons and ground-based observatories have probed the cosmic microwave background (CMB), the glow left over from 400,000 years after the big bang, for clues to the "shape" and composition of the cosmos. When the MAP data came out in February (*Science*, 14 February, p. 991), that era in cosmology ended. Scientists now know that the universe is geometrically "flat," that it is made up of about 27% matter and 73% dark energy, and that it is 13.7 billion years old. They have figured out, with great precision, how fast it is expanding. Now, they say, it's time to move on.

"I cannot overemphasize how important the MAP data is," says Max Tegmark, a cosmologist at the University of Pennsylvania in

Philadelphia. "But with MAP, it's not like it's all over and we should all switch into biophysics." On the contrary, he argues, "cosmology is becoming more and more fun." The pursuit of fun is taking scientists in two directions: forward in time from the CMB to glimpse surprising insights about when and how the first stars and galaxies were born; and backward, to give inflation—a class of theories that describe how the universe expanded less than  $10^{-32}$  seconds after the big bang—a long-awaited reality check. On both fronts, new observations of cosmic phenomena are beginning to take researchers into unexplored territory that the CMB alone could not reveal.

### Premature star birth

To illuminate this terra incognita, astronomers start with light. MAP's exquisite pictures of the CMB were photons bouncing off a cloud of hot gas, mostly hydrogen, that filled the universe when it was about 400,000 years old. During its 14-billion-year journey, stretched and cooled by the expanding fabric of the universe, the light traveled through other clouds of hydrogen. Early on, those clouds consisted largely of electrically neutral intact atoms that had condensed from

their component protons and electrons as the universe cooled. Later, energy from stars, galaxies, and other energetic cosmic objects stripped the electrons from the hydrogen nuclei, a process known as "reionization." Because neutral hydrogen is opaque to some wavelengths of light, determining how long neutral hydrogen "fog" suffused the universe before reionization burned it away should tell scientists when stars and galaxies ignited—a landmark in cosmic history.

By studying variations in the CMB's temperature and polarization, MAP astronomers figured out how much of the CMB had been absorbed by the neutral hydrogen fog during its long journey. To their surprise, their calculations showed that the fog began to burn off when the universe was a mere 200 million years old and then lifted rapidly. That implies that stars and galaxies must have been forming hundreds of millions of years earlier than most astronomers thought. "Literally, before 1 month ago, everyone agreed that reionization occurred at about [2 billion years after the big bang]," says Joe Silk, a cosmologist at the University of Oxford, U.K. "Something has to have been going on in protogalaxies."

To burn off fog so efficiently, says Columbia University physicist Zoltán Haiman, star formation in the early universe must have been radically different from what it is today. Martin Rees, Britain's Astronomer Royal, thinks that the first stars may have emitted much more hydrogen-ionizing ultraviolet (UV) radiation than present-day stars do. "Perhaps earlier stars were much more massive," he says. "They could have been 100

\* The Davis Meeting on Cosmic Inflation, 22 to 25 March.