Innovations

Packing it in
Chromos Molecular Systems Inc. & Athersys Inc.

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In the future, everyone will be boring. That was the surprising message from the science-fiction movie Gattaca, in which Ethan Hawke and Uma Thurman stumbled around a world in which genetically perfect humans are the norm.

Although Gattaca's script was profoundly snooze-inducing, its premise of widespread germline engineering is starting to look reasonable. "I certainly think it will happen," says Princeton geneticist Lee Silver. "The only question is when." Gregory Stock, director of the University of California, Los Angeles, Program on Medicine, Technology and Society, co-organized a 1998 symposium called "Engineering the Human Germline," the first major forum on human germline engineering. "The range of discussion," he says, "was whether it would happen in 20 years or 100 years, but not that it wouldn't occur."

The general public remains, for the most part, blissfully unaware of this impending revolution. But discussion in academia has started because the technologies for achieving germline engineering are rapidly being assembled. "So often technologies are created for one purpose and used for another," says Silver. This is certainly the case with germline engineering. Genes suitable for transfer are coming from the human genome project. Stem cells to receive the genes are being characterized by companies like Geron Corporation (Menlo Park, California) and Advanced Cell Technology, Inc. (Worcester, Massachusetts), ostensibly to create transplantable cells and tissues. And artificial minichromosomes to carry all the genes are being created by two companies — Chromos Molecular Systems Inc. (Burnaby, British Columbia, Canada) and Athersys Inc. (Cleveland, Ohio) — both of which swear they have no interest or intent in the area of germline engineering.

But others are showing plenty of interest. "The only way [germline engineering] will ever be significant is through the use of synthetic minichromosomes," says Stock. "This is the only way you can produce a technology that is sufficiently reproducible, safe and reliable."

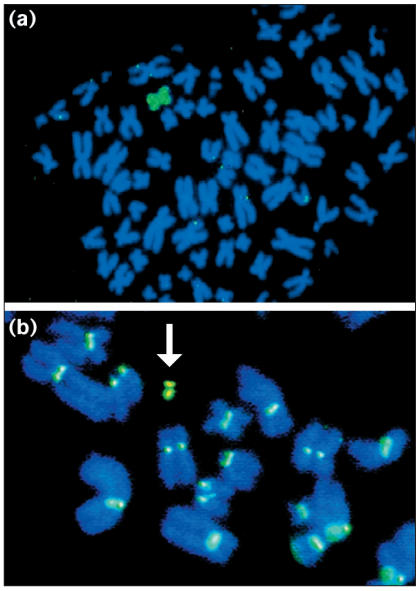
Artificial minichromosomes may end up as carriers for germline gene therapy.

An innocent start

Huntington Willard was and still is interested in something far less controversial than germline engineering. But to explore his interest — the basic requirements for human chromosome functioning and segregation — he wanted to make a synthetic human chromosome (Figure 1). Willard, now at Case Western Reserve University in Cleveland, Ohio, could only look on enviously in 1983 as Jack Szostak and Andrew Murray (then at Harvard Medical School in Boston, Massachusetts) put together selectable genes, origins of DNA replication, a centromere, and telomeres to create a yeast artificial chromosome (YAC).

For Willard, the stumbling block was the centromere — the DNA sequence that specifies attachment to the mitotic spindle. In budding yeast the centromere is a conserved 120 base pairs of DNA, but the search for a comparable sequence in humans was proving frustrating. By the late 1980s, Willard had started studying the repetitive satellite DNA characteristic of human centromeres, but "nobody at that time took seriously the idea that those were the centromere sequences," he says. Slowly satellite DNA gained favor, and in 1997 Willard published his killer result: transfection of a mixture of genomic DNA, alpha satellites and telomeres yielded lots of chromosome insertions and truncations, but at least one *de novo* synthetic minichromosome. Athersys had its founding technology.

Figure 1



Artificial chromosomes. **(a)** A mouse artificial chromosome (stained for mouse major satellite DNA, in green) created using the Chromos technology, in a spread of normal human chromosomes. **(b)** A smaller human synthetic chromosome created by Athersys. Images courtesy of Chromos and Athersys.

The Hungarian connection

In January of the same year, Chromos opened its doors in British Columbia. The company based itself on some rather obscure science from Gyula Hadlaczky (Hungarian Academy of Sciences, Szeged, Hungary). Hadlaczky had isolated a lambda clone of what he thought were candidate centromere sequences. He transfected the sequences into mouse cells and, lo and behold, got the

desired minichromosome. But the new chromosome had formed through an unusual pathway. Weak homology between the lambda sequence and rDNA near a centromere had targeted the introduced sequence to this region. Somehow this insertion activated a postulated megareplicator sequence. Amplification and centromere duplication formed a dicentric chromosome that broke, yielding a new minichromosome.

Chromos has repeated the procedure (although targeting now with rDNA) to generate 40–60 Mb chromosomes. "The event that we are triggering is reproducible, but we get different amplification events," says Chromos director of projects Carl Perez. The company now plans to add recombination sites for systems such as FLP/FRT or Cre/Lox to these existing chromosomes, and use the sites to shuttle in new genes. "That will speed up the production of a new chromosome from 6 months to 4–5 weeks," says Perez.

The first application for the chromosomes is protein production in cell culture, in a collaboration with Boehringer Ingelheim. The Chromos vector has allowed more rapid and extensive amplification of gene copy number and therefore protein output.

For production of therapeutic proteins in the milk of domestic animals, Perez hopes to beat the transgenic cloning crowd in cost and speed. To establish each new founder for cloning, a gene of interest must undergo random integration, which often leads to position effects that reduce transcription. The Chromos approach should avoid position effects, and allow the introduction of multiple, large genes. Mammary-specific expression can be pre-selected in mice. The cloners, meanwhile, cannot see expression of their genes in their transfected fetal cells, and so must wait to see if the promoter turns on in an expensive cloned cow.

Gene therapy, but somatic only, please

Chromos is interested initially in localized gene therapy for indications such as rheumatoid arthritis, using in-licensed genes that have yet to be identified. Delivery will be a challenge, but Chromos will try a combination of lipids and electro- or sonoporation.

Delivery problems may reflect difficulties in penetrating the cell, preventing DNA degradation, or establishing centromere proteins on naked DNA — a task that normal cells are never faced with. But artificial chromosomes offer the advantage, compared to viral gene delivery, of greater persistence for treating chronic diseases.

Athersys will focus initially on *ex vivo* gene therapy and is collaborating with Gene-Cell Inc. (Houston, Texas) to test a rapid microinjection delivery system. They expect to enter animal trials very soon, and the clinic in perhaps two years.

Early ambition

Athersys CEO Gil Van Bokkelen helped form the company out of Willard's laboratory, and gives the impression that his feverish energy has been driving it ever since. No warm-ups for Van Bokkelen; his first declaration after "hello" is to state that "our long-term strategic goal is to become a fully integrated company." The synthetic minichromosome technology, he says, "is something we are very excited about, but it's a relatively small part of the company."

A number of researchers, including Howard Cooke (Western General Hospital, Edinburgh), express doubts about the commercialization of the technology. "I think it's some way away from practical application," he says. The minichromosome technology has, however, moved significantly beyond the work described in Willard's 1997 publication. The transfection of unassembled DNA fragments "was a way to prove a point," says Van Bokkelen. "Now we work with fully defined pre-fabricated units." The assembly platform is bacterial artificial chromosomes (BACs), which Cooke is also investigating with Boehringer Ingelheim.

The future is Ethan

Chromos constructs are stable in both founder and progeny mice, but Perez says of human germ line gene therapy: "We wouldn't do it. It's not in our code of ethics to make a better person." Van Bokkelen is no less emphatic. "It's a no-no, an absolute mistake," he says.

Serious genetic diseases can increasingly be managed by embryo selection, they say, and no one has a good idea of how a human with an extra chromosome could mate with an unaltered human without causing genetic complications.

But those protestations and more were faced down by participants at the UCLA symposium. Mario Capecchi (University of Utah, Salt Lake City) proposed a recombination system to flip out the centromere in germ cells. "You wouldn't want to keep [the chromosomes] anyway if the technology is advancing," noted Stock.

But what we would we put in? "If you add in a new gene you don't really know how it will work," says Silver. "So you add in a gene that already exists, like a gene that some people have that gives HIV resistance. It's hard to imagine a government stopping parents giving their children something that other children already receive naturally."

These gene variants — including one that makes trained muscle more mechanically efficient (see *Nature*, **403**, p614) — are a start. But the real impetus, Silver says, will come with genes that reduce susceptibility to major diseases. It will take many years to accumulate these genes, says Silver, but when enough can be piled onto a high capacity artificial chromosome, parents will demand it. "If there are meaningful enhancements they will almost certainly be adopted by some people," says Stock. "And then there will be intense competitive pressure for the rest of us to join in."

Put on your suit, Ethan. We've arrived at Gattaca.

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